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(54) Title: ANTISENSE OLIGONUCLEOTIDE MODULATION OF TUMOR NECROSIS FACTOR- α (TNF- α) EXPRESSION			
(57) Abstract			
Compositions and methods are provided for inhibiting the expression of human tumor necrosis factor- α (TNF- α). Antisense oligonucleotides targeted to nucleic acids encoding TNF- α are preferred. Methods of using these oligonucleotides for inhibition of TNF- α expression and for treatment of diseases, particularly inflammatory and autoimmune diseases, associated with overexpression of TNF- α are provided.			

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**ANTISENSE OLIGONUCLEOTIDE MODULATION OF
TUMOR NECROSIS FACTOR- α (TNF- α) EXPRESSION**

5

This invention relates to compositions and methods for modulating expression of the human tumor necrosis factor- α (TNF- α) gene, which encodes a naturally present cytokine 10 involved in regulation of immune function and implicated in infectious and inflammatory disease. This invention is also directed to methods for inhibiting TNF- α mediated immune responses; these methods can be used diagnostically or therapeutically. Furthermore, this invention is 15 directed to treatment of conditions associated with expression of the human TNF- α gene.

BACKGROUND OF THE INVENTION

Tumor necrosis factor α (TNF- α also cachectin) is an important cytokine that plays a role in host defense. The 20 cytokine is produced primarily in macrophages and monocytes in response to infection, invasion, injury, or inflammation. Some examples of inducers of TNF- α include bacterial endotoxins, bacteria, viruses, lipopolysaccharide (LPS) and cytokines including GM-CSF, IL-1, IL-2 and IFN- γ .

25 TNF- α interacts with two different receptors, TNF receptor I (TNFRI, p55) and TNFRII (p75), in order to transduce its effects, the net result of which is altered gene expression. Cellular factors induced by TNF- α include interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 30 (IL-8), interferon- γ (IFN- γ), platelet derived growth factor (PDGF) and epidermal growth factor (EGF), and endothelial cell adhesion molecules including endothelial leukocyte

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adhesion molecule 1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Tracey,K.J., et al., *Annu. Rev. Cell Biol.*, 1993, 9, 317-343; Arvin,B., et al., *Ann. NY Acad. Sci.*, 1995, 765, 62-71).

5 Despite the protective effects of the cytokine, overexpression of TNF- α often results in disease states, particularly in infectious, inflammatory and autoimmune diseases. This process may involve the apoptotic pathways 10 (Ksontini,R., et al., *J. Immunol.*, 1998, 160, 4082-4089).

High levels of plasma TNF- α have been found in infectious diseases such as sepsis syndrome, bacterial meningitis, cerebral malaria, and AIDS; autoimmune diseases such as 15 rheumatoid arthritis, inflammatory bowel disease (including Crohn's disease), sarcoidosis, multiple sclerosis, Kawasaki syndrome, graft-versus-host disease and transplant (allograft) rejection; and organ failure conditions such as adult respiratory distress syndrome, congestive heart failure, acute liver failure and myocardial infarction 20 (Eigler,A., et al., *Immunol. Today*, 1997, 18, 487-492).

Other diseases in which TNF- α is involved include asthma (Shah,A., et al., *Clinical and Experimental Allergy*, 1995, 25, 1038-1044), brain injury following ischemia (Arvin,B., et al., *Ann. NY Acad. Sci.*, 1995, 765, 62-71), non-insulin-dependent diabetes mellitus (Hotamisligil,G.S., et al., *Science*, 1993, 259, 87-90), insulin-dependent diabetes 25 mellitus (Yang,X.-D., et al., *J. Exp. Med.*, 1994, 180, 995-1004), hepatitis (Ksontini,R., et al., *J. Immunol.*, 1998, 160, 4082-4089), atopic dermatitis (Sumimoto,S., et al., *Arch. Dis. Child.*, 1992, 67, 277-279), and pancreatitis 30 (Norman,J.G., et al., *Surgery*, 1996, 120, 515-521).

Further, inhibitors of TNF- α have been suggested to be useful for cancer prevention (Suganuma,M., et al. (*Cancer*

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Res., 1996, 56, 3711-3715). Elevated TNF- α expression may also play a role in obesity (Kern, P.A., J. Nutr., 1997, 127, 1917S-1922S). TNF- α was found to be expressed in human adipocytes and increased expression, in general, 5 correlated with obesity.

There are currently several approaches to inhibiting TNF- α expression. Approaches used to treat rheumatoid arthritis include a chimeric anti-TNF- α antibody, a humanized monoclonal anti-TNF- α antibody, and recombinant 10 human soluble TNF- α receptor (Camussi, G., Drugs, 1998, 55, 613-620). Other examples are indirect TNF- α inhibitors including phosphodiesterase inhibitors (e.g. pentoxifylline) and metalloprotease inhibitors (Eigler, A., et al., Immunol. Today, 1997, 18, 487-492). An additional 15 class of direct TNF- α inhibitors is oligonucleotides, including triplex-forming oligonucleotides, ribozymes, and antisense oligonucleotides.

Several publications describe the use of oligonucleotides targeting TNF- α by non-antisense 20 mechanisms. U.S. Patent 5,650,316, WO 95/33493 and Aggarwal, B.B. et al. (Cancer Research, 1996, 56, 5156-5164) disclose triplex-forming oligonucleotides targeting TNF- α . WO 95/32628 discloses triplex-forming oligonucleotides especially those possessing one or more stretches of 25 guanosine residues capable of forming secondary structure. WO 94/10301 discloses ribozyme compounds active against TNF- α mRNA. WO 95/23225 discloses enzymatic nucleic acid molecules active against TNF- α mRNA.

A number of publications have described the use of 30 antisense oligonucleotides targeting nucleic acids encoding TNF- α . The TNF- α gene has four exons and three introns. WO 93/09813 discloses TNF- α antisense oligonucleotides

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conjugated to a radioactive moiety, including sequences targeted to the 5'-UTR, AUG start site, exon 1, and exon 4 including the stop codon of human TNF- α . EP 0 414 607 B1 discloses antisense oligonucleotides targeting the AUG
5 start codon of human TNF- α . WO 95/00103 claims antisense oligonucleotides to human TNF- α including sequences targeted to exon 1 including the AUG start site.

Hartmann, G. et al. (*Mol. Med.*, 1996, 2, 429-438) disclose uniform phosphorothioates and mixed backbone

10 phosphorothioate/ phosphodiester oligonucleotides targeted to the AUG start site of human TNF- α . Hartmann, G. et al. (*Antisense Nucleic Acid Drug Devel.*, 1996, 6, 291-299) disclose antisense phosphorothioate oligonucleotides targeted to the AUG start site, the exon 1/intron 1

15 junction, and exon 4 of human TNF- α . d'Hellencourt, C.F. et al. (*Biochim. Biophys. Acta*, 1996, 1317, 168-174) designed and tested a series of unmodified oligonucleotides targeted to the 5'-UTR, and exon 1, including the AUG start site, of human TNF- α . Additionally, one oligonucleotide each was

20 targeted to exon 4 and the 3'-UTR of human TNF- α and one oligonucleotide was targeted to the AUG start site of mouse TNF- α . Rojanasakul, Y. et al. (*J. Biol. Chem.*, 1997, 272, 3910-3914) disclose an antisense phosphorothioate oligonucleotide targeted to the AUG start site of mouse

25 TNF- α . Taylor, M.F. et al. (*J. Biol. Chem.*, 1996, 271, 17445-17452 and *Antisense Nucleic Acid Drug Devel.*, 1998, 8, 199-205) disclose morpholino, methyl-morpholino, phosphodiester and phosphorothioate oligonucleotides targeted to the 5'-UTR and AUG start codon of mouse TNF- α .

30 Tu, G.-C. et al. (*J. Biol. Chem.*, 1998, 273, 25125-25131) designed and tested 42 phosphorothioate oligonucleotides targeting sequences throughout the rat TNF- α gene.

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Interestingly, some phosphorothioate oligodeoxynucleotides have been found to enhance lipopolysaccharide-stimulated TNF- α synthesis up to four fold due to nonspecific immunostimulatory effects (Hartmann et al. Mol. Med., 1996, 2, 429-438).

Accordingly, there remains an unmet need for therapeutic compositions and methods for inhibiting expression of TNF- α , and disease processes associated therewith.

10 **BRIEF DESCRIPTION OF THE INVENTION**

The present invention provides oligonucleotides which are targeted to nucleic acids encoding TNF- α and are capable of modulating TNF- α expression. The present invention also provides chimeric oligonucleotides targeted 15 to nucleic acids encoding human TNF- α . The oligonucleotides of the invention are believed to be useful both diagnostically and therapeutically, and are believed to be particularly useful in the methods of the present invention.

20 The present invention also comprises methods of modulating the expression of human TNF- α , in cells and tissues, using the oligonucleotides of the invention. Methods of inhibiting TNF- α expression are provided; these methods are believed to be useful both therapeutically and 25 diagnostically. These methods are also useful as tools, for example, for detecting and determining the role of TNF- α in various cell functions and physiological processes and conditions and for diagnosing conditions associated with expression of TNF- α .

30 The present invention also comprises methods for diagnosing and treating infectious and inflammatory diseases, particularly diabetes, rheumatoid arthritis, Crohn's disease, pancreatitis, multiple sclerosis, atopic

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dermatitis and hepatitis. These methods are believed to be useful, for example, in diagnosing TNF- α -associated disease progression. These methods employ the oligonucleotides of the invention. These methods are believed to be useful both therapeutically, including prophylactically, and as clinical research and diagnostic tools.

DETAILED DESCRIPTION OF THE INVENTION

TNF- α plays an important regulatory role in the immune response to various foreign agents. Overexpression of TNF- α results in a number of infectious and inflammatory diseases. As such, this cytokine represents an attractive target for treatment of such diseases. In particular, modulation of the expression of TNF- α may be useful for the treatment of diseases such as Crohn's disease, diabetes mellitus, multiple sclerosis, rheumatoid arthritis, hepatitis, pancreatitis and asthma.

The present invention employs antisense compounds, particularly oligonucleotides, for use in modulating the function of nucleic acid molecules encoding TNF- α , ultimately modulating the amount of TNF- α produced. This is accomplished by providing oligonucleotides which specifically hybridize with nucleic acids, preferably mRNA, encoding TNF- α .

This relationship between an antisense compound such as an oligonucleotide and its complementary nucleic acid target, to which it hybridizes, is commonly referred to as "antisense". "Targeting" an oligonucleotide to a chosen nucleic acid target, in the context of this invention, is a multistep process. The process usually begins with identifying a nucleic acid sequence whose function is to be modulated. This may be, as examples, a cellular gene (or mRNA made from the gene) whose expression is associated with a particular disease state, or a foreign nucleic acid

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from an infectious agent. In the present invention, the targets are nucleic acids encoding TNF- α ; in other words, a gene encoding TNF- α , or mRNA expressed from the TNF- α gene. mRNA which encodes TNF- α is presently the preferred target.

5 The targeting process also includes determination of a site or sites within the nucleic acid sequence for the antisense interaction to occur such that modulation of gene expression will result.

In accordance with this invention, persons of ordinary skill in the art will understand that messenger RNA includes not only the information to encode a protein using the three letter genetic code, but also associated ribonucleotides which form a region known to such persons as the 5'-untranslated region, the 3'-untranslated region, 15 the 5' cap region and intron/exon junction ribonucleotides. Thus, oligonucleotides may be formulated in accordance with this invention which are targeted wholly or in part to these associated ribonucleotides as well as to the informational ribonucleotides. The oligonucleotide may 20 therefore be specifically hybridizable with a transcription initiation site region, a translation initiation codon region, a 5' cap region, an intron/exon junction, coding sequences, a translation termination codon region or sequences in the 5'- or 3'-untranslated region. Since, as 25 is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon." A minority of genes have 30 a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function *in vivo*. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator

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amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which 5 may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used *in vivo* to initiate 10 translation of an mRNA molecule transcribed from a gene encoding TNF- α , regardless of the sequence(s) of such codons. It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the 15 corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively). The terms "start codon region," "AUG region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either 20 direction (i.e., 5' or 3') from a translation initiation codon. This region is a preferred target region. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 25 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon. This region is a preferred target region. The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the 30 translation termination codon, is also a region which may be targeted effectively. Other preferred target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including 35 nucleotides between the 5' cap site and the translation

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initiation codon of an mRNA or corresponding nucleotides on the gene and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus 5 including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an 10 mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap. The 5' cap region may also be a preferred target region.

Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as 15 "introns," which are excised from a pre-mRNA transcript to yield one or more mature mRNAs. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. mRNA splice sites, i.e., exon-exon or intron-exon junctions, may 20 also be preferred target regions, and are particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular mRNA splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also 25 preferred targets. Targeting particular exons in alternatively spliced mRNAs may also be preferred. It has also been found that introns can also be effective, and therefore preferred, target regions for antisense compounds targeted, for example, to DNA or pre-mRNA.

30 Once the target site or sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired modulation.

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"Hybridization", in the context of this invention, means hydrogen bonding, also known as Watson-Crick base pairing, between complementary bases, usually on opposite nucleic acid strands or two regions of a nucleic acid strand. Guanine and cytosine are examples of complementary bases which are known to form three hydrogen bonds between them. Adenine and thymine are examples of complementary bases which form two hydrogen bonds between them.

"Specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity such that stable and specific binding occurs between the DNA or RNA target and the oligonucleotide.

It is understood that an oligonucleotide need not be 100% complementary to its target nucleic acid sequence to be specifically hybridizable. An oligonucleotide is specifically hybridizable when binding of the oligonucleotide to the target interferes with the normal function of the target molecule to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment or, in the case of *in vitro* assays, under conditions in which the assays are conducted.

Hybridization of antisense oligonucleotides with mRNA interferes with one or more of the normal functions of mRNA. The functions of mRNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in by the RNA. Binding of specific protein(s) to

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the RNA may also be interfered with by antisense oligonucleotide hybridization to the RNA.

The overall effect of interference with mRNA function is modulation of expression of TNF- α . In the context of 5 this invention "modulation" means either inhibition or stimulation; i.e., either a decrease or increase in expression. This modulation can be measured in ways which are routine in the art, for example by Northern blot assay of mRNA expression, or reverse transcriptase PCR, as taught 10 in the examples of the instant application or by Western blot or ELISA assay of protein expression, or by an immunoprecipitation assay of protein expression. Effects of antisense oligonucleotides of the present invention on TNF- α expression can also be determined as taught in the 15 examples of the instant application. Inhibition is presently a preferred form of modulation.

The oligonucleotides of this invention can be used in diagnostics, therapeutics, prophylaxis, and as research reagents and in kits. Since the oligonucleotides of this 20 invention hybridize to nucleic acids encoding TNF- α , sandwich, colorimetric and other assays can easily be constructed to exploit this fact. Provision of means for detecting hybridization of oligonucleotides with the TNF- α gene or mRNA can routinely be accomplished. Such provision 25 may include enzyme conjugation, radiolabelling or any other suitable detection systems. Kits for detecting the presence or absence of TNF- α may also be prepared.

The present invention is also suitable for diagnosing abnormal inflammatory states in tissue or other samples 30 from patients suspected of having an inflammatory disease such as rheumatoid arthritis. The ability of the oligonucleotides of the present invention to inhibit inflammatory processes may be employed to diagnose such states. A number of assays may be formulated employing the

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present invention, which assays will commonly comprise contacting a tissue sample with an oligonucleotide of the invention under conditions selected to permit detection and, usually, quantitation of such inhibition. In the 5 context of this invention, to "contact" tissues or cells with an oligonucleotide or oligonucleotides means to add the oligonucleotide(s), usually in a liquid carrier, to a cell suspension or tissue sample, either *in vitro* or *ex vivo*, or to administer the oligonucleotide(s) to cells or 10 tissues within an animal.

The oligonucleotides of this invention may also be used for research purposes. Thus, the specific hybridization exhibited by the oligonucleotides may be used for assays, purifications, cellular product preparations 15 and in other methodologies which may be appreciated by persons of ordinary skill in the art.

In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid. This term 20 includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent intersugar (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over 25 native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced binding to target and increased stability in the presence of nucleases.

The antisense compounds in accordance with this 30 invention preferably comprise from about 5 to about 50 nucleobases. Particularly preferred are antisense oligonucleotides comprising from about 8 to about 30 nucleobases (i.e. from about 8 to about 30 linked nucleosides). As is known in the art, a nucleoside is a 35 base-sugar combination. The base portion of the nucleoside

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is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn the respective ends of this linear polymeric structure can be further joined to form a circular structure, however, open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkyl-phosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates,

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thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to U.S. Patent 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050.

Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patent 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967;

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5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240;
5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704;
5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439.

In other preferred oligonucleotide mimetics, both the
5 sugar and the internucleoside linkage, i.e., the backbone,
of the nucleotide units are replaced with novel groups.
The base units are maintained for hybridization with an
appropriate nucleic acid target compound. One such
10 oligomeric compound, an oligonucleotide mimetic that has
been shown to have excellent hybridization properties, is
referred to as a peptide nucleic acid (PNA). In PNA
compounds, the sugar-backbone of an oligonucleotide is
replaced with an amide containing backbone, in particular
an aminoethylglycine backbone. The nucleobases are
15 retained and are bound directly or indirectly to aza
nitrogen atoms of the amide portion of the backbone.
Representative United States patents that teach the
preparation of PNA compounds include, but are not limited
to, U.S.: 5,539,082; 5,714,331; and 5,719,262. Further
20 teaching of PNA compounds can be found in Nielsen et al.
(Science, 1991, 254, 1497-1500).

Most preferred embodiments of the invention are
oligonucleotides with phosphorothioate backbones and
oligonucleosides with heteroatom backbones, and in
25 particular -CH₂-NH-O-CH₂-, -CH₂-N(CH₃)-O-CH₂- [known as a
methylene (methylimino) or MMI backbone], -CH₂-O-N(CH₃)-CH₂-
, -CH₂-N(CH₃)-N(CH₃)-CH₂- and -O-N(CH₃)-CH₂-CH₂- [wherein the
native phosphodiester backbone is represented as -O-P-O-
CH₂-] of the above referenced U.S. Patent 5,489,677, and the
30 amide backbones of the above referenced U.S. Patent
5,602,240. Also preferred are oligonucleotides having
morpholino backbone structures of the above-referenced U.S.
patent 5,034,506.

Modified oligonucleotides may also contain one or more
35 substituted sugar moieties. Preferred oligonucleotides

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comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl, O-alkyl-O-alkyl, O-, S-, or N-alkenyl, or O-, S- or N-alkynyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Particularly preferred are
5 O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃, O(CH₂)₂ON(CH₃)₂, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C₁ to C₁₀
10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group,
15 an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al.,
20 *Helv. Chim. Acta* 1995, 78, 486-504) i.e., an alkoxyalkoxy group.
Other preferred modifications include 2'-methoxy (2'-O-CH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F). Similar modifications may also be made at other
25 positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugars structures include, but are not limited to, U.S. Patent
30 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878;
35 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811;

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5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053;
5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920.

Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C or m5c), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Patent 3,687,808, those disclosed in the *Concise Encyclopedia Of Polymer Science And Engineering 1990*, pages 858-859, Kroschwitz, J.I., ed. John Wiley & Sons, those disclosed by Englisch et al. (*Angewandte Chemie, International Edition 1991*, 30, 613-722), and those disclosed by Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., *Antisense Research and Applications 1993*, CRC Press, Boca Raton, pages 289-302. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-

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propynylcytosine. 5-Methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., *Antisense Research and Applications* 1993, CRC Press, Boca Raton, pages 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Patent 3,687,808, as well as U.S. Patent 4,845,205; 5,130,302; 10 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 15 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941.

Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA* 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.* 1994, 4, 1053-1059), a thioether, e.g., hexyl-20 25 *S*-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.* 1992, 660, 306-309; Manoharan et al., *Bioorg. Med. Chem. Let.* 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.* 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et 30 35 al., *EMBO J.* 1991, 10, 1111-1118; Kabanov et al., *FEBS Lett.* 1990, 259, 327-330; Svinarchuk et al., *Biochimie* 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron*

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Lett. 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res. 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett. 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther. 1996, 277, 923-937).

10 Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S. Patent 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 15 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 20 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 25 5,599,928 and 5,688,941.

25 The present invention also includes oligonucleotides which are chimeric oligonucleotides. "Chimeric" oligonucleotides or "chimeras," in the context of this invention, are oligonucleotides which contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at 30 least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An

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additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of antisense inhibition of gene expression.

5 Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art. This RNase H-mediated cleavage of the RNA target is distinct from the use of ribozymes to cleave nucleic acids. Ribozymes are not comprehended by the present invention.

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Examples of chimeric oligonucleotides include but are not limited to "gapmers," in which three distinct regions are present, normally with a central region flanked by two regions which are chemically equivalent to each other but distinct from the gap. A preferred example of a gapmer is an oligonucleotide in which a central portion (the "gap") of the oligonucleotide serves as a substrate for RNase H and is preferably composed of 2'-deoxynucleotides, while the flanking portions (the 5' and 3' "wings") are modified to have greater affinity for the target RNA molecule but are unable to support nuclease activity (e.g., fluoro- or 2'-O-methoxyethyl-substituted). Chimeric oligonucleotides are not limited to those with modifications on the sugar, but may also include oligonucleosides or oligonucleotides with modified backbones, e.g., with regions of phosphorothioate (P=S) and phosphodiester (P=O) backbone linkages or with regions of MMI and P=S backbone linkages. Other chimeras include "wingmers," also known in the art as "hemimers," that is, oligonucleotides with two distinct regions. In a preferred example of a wingmer, the 5' portion of the oligonucleotide serves as a substrate for RNase H and is preferably composed of 2'-deoxynucleotides,

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whereas the 3' portion is modified in such a fashion so as to have greater affinity for the target RNA molecule but is unable to support nuclease activity (e.g., 2'-fluoro- or 2'-O-methoxyethyl- substituted), or vice-versa. In one 5 embodiment, the oligonucleotides of the present invention contain a 2'-O-methoxyethyl (2'-O-CH₂CH₂OCH₃) modification on the sugar moiety of at least one nucleotide. This 10 modification has been shown to increase both affinity of the oligonucleotide for its target and nuclease resistance 15 of the oligonucleotide. According to the invention, one, a plurality, or all of the nucleotide subunits of the oligonucleotides of the invention may bear a 2'-O- methoxyethyl (-O-CH₂CH₂OCH₃) modification. Oligonucleotides comprising a plurality of nucleotide subunits having a 2'-O-methoxyethyl modification can have such a modification on any of the nucleotide subunits within the oligonucleotide, and may be chimeric oligonucleotides. Aside from or in addition to 2'-O-methoxyethyl modifications, 20 oligonucleotides containing other modifications which enhance antisense efficacy, potency or target affinity are also preferred. Chimeric oligonucleotides comprising one or more such modifications are presently preferred.

The oligonucleotides used in accordance with this invention may be conveniently and routinely made through 25 the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the talents of the 30 routineer. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and 2'-alkoxy or 2'-alkoxyalkoxy derivatives, including 2'-O- methoxyethyl oligonucleotides (Martin, P., *Helv. Chim. Acta* 1995, 78, 486-504). It is also well known to use similar 35 techniques and commercially available modified amidites and

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controlled-pore glass (CPG) products such as biotin, fluorescein, acridine or psoralen-modified amidites and/or CPG (available from Glen Research, Sterling, VA) to synthesize fluorescently labeled, biotinylated or other 5 conjugated oligonucleotides.

The antisense compounds of the present invention include bioequivalent compounds, including pharmaceutically acceptable salts and prodrugs. This is intended to encompass any pharmaceutically acceptable salts, esters, or 10 salts of such esters, or any other compound which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically 15 acceptable salts of the nucleic acids of the invention and prodrugs of such nucleic acids. "Pharmaceutically acceptable salts" are physiologically and pharmaceutically acceptable salts of the nucleic acids of the invention: i.e., salts that retain the desired biological activity of 20 the parent compound and do not impart undesired toxicological effects thereto (see, for example, Berge et al., "Pharmaceutical Salts," *J. of Pharma Sci.* 1977, 66, 1-19).

For oligonucleotides, examples of pharmaceutically 25 acceptable salts include but are not limited to (a) salts formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamines such as spermine and spermidine, etc.; (b) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic 30 acid, sulfuric acid, phosphoric acid, nitric acid and the like; © salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, 35 palmitic acid, alginic acid, polyglutamic acid,

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naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (d) salts formed from elemental anions such as chlorine, bromine, and
5 iodine.

The oligonucleotides of the invention may additionally or alternatively be prepared to be delivered in a "prodrug" form. The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form that is converted to
10 an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the oligonucleotides of the invention are prepared as SATE [(S-acetyl-2-thioethyl) phosphate]
15 derivatives according to the methods disclosed in WO 93/24510.

For therapeutic or prophylactic treatment, oligonucleotides are administered in accordance with this invention. Oligonucleotide compounds of the invention may
20 be formulated in a pharmaceutical composition, which may include pharmaceutically acceptable carriers, thickeners, diluents, buffers, preservatives, surface active agents, neutral or cationic lipids, lipid complexes, liposomes, penetration enhancers, carrier compounds and other
25 pharmaceutically acceptable carriers or excipients and the like in addition to the oligonucleotide. Such compositions and formulations are comprehended by the present invention.

Pharmaceutical compositions comprising the oligonucleotides of the present invention may include
30 penetration enhancers in order to enhance the alimentary delivery of the oligonucleotides. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., fatty acids, bile salts, chelating agents, surfactants and non-surfactants (Lee et al., *Critical
35 Reviews in Therapeutic Drug Carrier Systems* 1991, 8,

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91-192; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems* 1990, 7, 1-33). One or more penetration enhancers from one or more of these broad categories may be included. Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, recinleate, monoolein (a.k.a. 5 1-monoleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glyceryl 1-monocaprate, 10 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, mono- and di-glycerides and physiologically acceptable salts thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et 15 al., *Critical Reviews in Therapeutic Drug Carrier Systems* 1991, page 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems* 1990, 7, 1; El-Hariri et al., *J. Pharm. Pharmacol.* 1992 44, 651-654).

The physiological roles of bile include the 20 facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 *In: Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman et al., eds., McGraw-Hill, New York, NY, 1996, pages 934-935). Various natural bile salts, and their 25 synthetic derivatives, act as penetration enhancers. Thus, the term "bile salt" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives.

Complex formulations comprising one or more 30 penetration enhancers may be used. For example, bile salts may be used in combination with fatty acids to make complex formulations.

Chelating agents include, but are not limited to, disodium ethylenediaminetetraacetate (EDTA), citric acid,

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salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems* 1991, page 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems* 1990, 7, 1-33; Buur et al., *J. Control Rel.* 1990, 14, 43-51). Chelating agents have the added advantage of also serving as DNase inhibitors.

Surfactants include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems* 1991, page 92); and perfluorochemical emulsions, such as FC-43 (Takahashi et al., *J. Pharm. Pharmacol.* 1988, 40, 252-257).

Non-surfactants include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems* 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., *J. Pharm. Pharmacol.* 1987, 39, 621-626).

As used herein, "carrier compound" refers to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity *per se*) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation.

The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition

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between the carrier compound and the nucleic acid for a common receptor. In contrast to a carrier compound, a "pharmaceutically acceptable carrier" (excipient) is a pharmaceutically acceptable solvent, suspending agent or 5 any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The pharmaceutically acceptable carrier may be liquid or solid and is selected with the planned manner of administration in mind so as to provide for the desired bulk, consistency, 10 etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutically acceptable carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl 15 methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic 20 acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrates (e.g., starch, sodium starch glycolate, etc.); or wetting agents (e.g., sodium lauryl sulphate, etc.). Sustained release oral delivery systems 25 and/or enteric coatings for orally administered dosage forms are described in U.S. Patents 4,704,295; 4,556,552; 4,309,406; and 4,309,404.

The compositions of the present invention may 30 additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional compatible pharmaceutically-active materials such as, e.g., antipruritics, astringents, local anesthetics or 35 anti-inflammatory agents, or may contain additional

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materials useful in physically formulating various dosage forms of the compositions of present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However,
5 such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the invention.

Regardless of the method by which the oligonucleotides of the invention are introduced into a patient, colloidal dispersion systems may be used as delivery vehicles to enhance the *in vivo* stability of the oligonucleotides and/or to target the oligonucleotides to a particular organ, tissue or cell type. Colloidal dispersion systems include, but are not limited to, macromolecule complexes,
10 nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, liposomes and lipid:oligonucleotide complexes of uncharacterized structure. A preferred colloidal dispersion system is a plurality of liposomes. Liposomes
15 are microscopic spheres having an aqueous core surrounded by one or more outer layers made up of lipids arranged in a bilayer configuration (see, generally, Chonn *et al.*,
20 *Current Op. Biotech.* 1995, 6, 698-708).

The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, epidermal, and transdermal), oral or parenteral.
25 Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal or intramuscular injection, pulmonary administration, e.g., by inhalation or insufflation, or intracranial, e.g., intrathecal or intraventricular, administration. Oligonucleotides with at
30 least one 2'-O-methoxyethyl modification are believed to be

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particularly useful for oral administration.

Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders.

5 Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

10 Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

15 Compositions for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. In some cases it may be more effective to treat a patient with an oligonucleotide of the invention in conjunction with other traditional therapeutic modalities in order to increase the 20 efficacy of a treatment regimen. In the context of the invention, the term "treatment regimen" is meant to encompass therapeutic, palliative and prophylactic modalities. For example, a patient may be treated with conventional chemotherapeutic agents such as those used for 25 tumor and cancer treatment. When used with the compounds of the invention, such chemotherapeutic agents may be used individually, sequentially, or in combination with one or more other such chemotherapeutic agents.

30 The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution 35 of the disease state is achieved. Optimal dosing schedules

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can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on
5 the relative potency of individual oligonucleotides, and can generally be estimated based on EC₅₀s found to be effective *in vitro* and in *in vivo* animal models. In general, dosage is from 0.01 µg to 100 g per kg of body weight, and may be given once or more daily, weekly,
10 monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be
15 desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 µg to 100 g per kg of body weight, once or more daily, to once every 20 years.

20 Thus, in the context of this invention, by "therapeutically effective amount" is meant the amount of the compound which is required to have a therapeutic effect on the treated individual. This amount, which will be apparent to the skilled artisan, will depend upon the age
25 and weight of the individual, the type of disease to be treated, perhaps even the gender of the individual, and other factors which are routinely taken into consideration when designing a drug treatment. A therapeutic effect is assessed in the individual by measuring the effect of the
30 compound on the disease state in the animal.

The following examples illustrate the present invention and are not intended to limit the same.

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EXAMPLES

EXAMPLE 1: Synthesis of Oligonucleotides

Unmodified oligodeoxynucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine. β -cyanoethyldiisopropyl-phosphoramidites are purchased from Applied Biosystems (Foster City, CA). For phosphorothioate oligonucleotides, the standard oxidation bottle was replaced by a 0.2 M solution of ^3H -1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation cycle wait step was increased to 68 seconds and was followed by the capping step. Cytosines may be 5-methyl cytosines. (5-methyl deoxycytidine phosphoramidites available from Glen Research, Sterling, VA or Amersham Pharmacia Biotech, Piscataway, NJ)

2'-methoxy oligonucleotides are synthesized using 2'-methoxy β -cyanoethyldiisopropyl-phosphoramidites (Chemgenes, Needham, MA) and the standard cycle for unmodified oligonucleotides, except the wait step after pulse delivery of tetrazole and base is increased to 360 seconds. Other 2'-alkoxy oligonucleotides are synthesized by a modification of this method, using appropriate 2'-modified amidites such as those available from Glen Research, Inc., Sterling, VA.

2'-fluoro oligonucleotides are synthesized as described in Kawasaki et al. (*J. Med. Chem.* 1993, 36, 831-841). Briefly, the protected nucleoside N⁶-benzoyl-2'-deoxy-2'-fluoroadenosine is synthesized utilizing commercially available 9- β -D-arabinofuranosyladenine as starting material and by modifying literature procedures whereby the 2'- α -fluoro atom is introduced by a S_N2-displacement of a 2'- β -O-trifyl group. Thus N⁶-benzoyl-9- β -D-arabinofuranosyladenine is selectively protected in

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moderate yield as the 3',5'-ditetrahydropyranyl (THP) intermediate. Deprotection of the THP and N⁶-benzoyl groups is accomplished using standard methodologies. Standard methods are also used to obtain the 5'-dimethoxytrityl-
5 (DMT) and 5'-DMT-3'-phosphoramidite intermediates.

The synthesis of 2'-deoxy-2'-fluoroguanosine is accomplished using tetraisopropylidisiloxanyl (TPDS) protected 9-β-D-arabinofuranosylguanine as starting material, and conversion to the intermediate diisobutyryl-
10 arabinofuranosylguanosine. Deprotection of the TPDS group is followed by protection of the hydroxyl group with THP to give diisobutyryl di-THP protected arabinofuranosylguanine. Selective O-deacylation and triflation is followed by treatment of the crude product with fluoride, then
15 deprotection of the THP groups. Standard methodologies are used to obtain the 5'-DMT- and 5'-DMT-3'-phosphoramidites.

Synthesis of 2'-deoxy-2'-fluorouridine is accomplished by the modification of a known procedure in which 2, 2'-anhydro-1-β-D-arabinofuranosyluracil is treated with 70%
20 hydrogen fluoride-pyridine. Standard procedures are used to obtain the 5'-DMT and 5'-DMT-3' phosphoramidites.

2'-deoxy-2'-fluorocytidine is synthesized via amination of 2'-deoxy-2'-fluorouridine, followed by selective protection to give N⁴-benzoyl-2'-deoxy-2'-
25 fluorocytidine. Standard procedures are used to obtain the 5'-DMT and 5'-DMT-3' phosphoramidites.

2'-(2-methoxyethyl)-modified amidites were synthesized according to Martin, P. (*Helv. Chim. Acta* 1995, 78, 486-
30 506). For ease of synthesis, the last nucleotide may be a deoxynucleotide. 2'-O-CH₂CH₂OCH₃.cytosines may be 5-methyl cytosines.

*Synthesis of 5-Methyl cytosine monomers:*2,2'-Anhydro[1-(β -D-arabinofuranosyl)-5-methyluridinel:

5 5-Methyluridine (ribosylthymine, commercially available through Yamasa, Choshi, Japan) (72.0 g, 0.279 M), diphenylcarbonate (90.0 g, 0.420 M) and sodium bicarbonate (2.0 g, 0.024 M) were added to DMF (300 mL). The mixture was heated to reflux, with stirring, allowing the evolved carbon dioxide gas to be released in a controlled manner.

10 After 1 hour, the slightly darkened solution was concentrated under reduced pressure. The resulting syrup was poured into diethylether (2.5 L), with stirring. The product formed a gum. The ether was decanted and the residue was dissolved in a minimum amount of methanol (ca. 15 400 mL). The solution was poured into fresh ether (2.5 L) to yield a stiff gum. The ether was decanted and the gum was dried in a vacuum oven (60°C at 1 mm Hg for 24 hours) to give a solid which was crushed to a light tan powder (57 g, 85% crude yield). The material was used as is for further 20 reactions.

2'-O-Methoxyethyl-5-methyluridine:

25 2,2'-Anhydro-5-methyluridine (195 g, 0.81 M), tris(2-methoxyethyl)borate (231 g, 0.98 M) and 2-methoxyethanol (1.2 L) were added to a 2 L stainless steel pressure vessel and placed in a pre-heated oil bath at 160°C. After heating for 48 hours at 155-160°C, the vessel was opened and the solution evaporated to dryness and triturated with MeOH (200 mL). The residue was suspended in hot acetone (1 L). The insoluble salts were filtered, washed with acetone (150 mL) and the filtrate evaporated. The residue (280 g) was dissolved in CH₃CN (600 mL) and evaporated. A silica gel column (3 kg) was packed in CH₂Cl₂/acetone/MeOH (20:5:3) containing 0.5% Et₃NH. The residue was dissolved in CH₂Cl₂ (250 mL) and adsorbed onto silica (150 g) prior to loading 30 onto the column. The product was eluted with the packing 35

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solvent to give 160 g (63%) of product.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine:

2'-O-Methoxyethyl-5-methyluridine (160 g, 0.506 M) was co-evaporated with pyridine (250 mL) and the dried residue dissolved in pyridine (1.3 L). A first aliquot of di-

5 methoxytrityl chloride (94.3 g, 0.278 M) was added and the mixture stirred at room temperature for one hour. A second aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the reaction stirred for an additional one hour.

10 Methanol (170 mL) was then added to stop the reaction.

HPLC showed the presence of approximately 70% product. The solvent was evaporated and triturated with CH₃CN (200 mL).

The residue was dissolved in CHCl₃ (1.5 L) and extracted with 2x500 mL of saturated NaHCO₃ and 2x500 mL of saturated

15 NaCl. The organic phase was dried over Na₂SO₄, filtered and evaporated. 275 g of residue was obtained. The residue was purified on a 3.5 kg silica gel column, packed and eluted with EtOAc/Hexane/Acetone (5:5:1) containing 0.5% Et₃NH. The pure fractions were evaporated to give 164 g of

20 product. Approximately 20 g additional was obtained from the impure fractions to give a total yield of 183 g (57%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine:

25 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (106 g, 0.167 M), DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of pyridine) and acetic anhydride (24.38 mL, 0.258 M) were combined and

stirred at room temperature for 24 hours. The reaction was monitored by tlc by first quenching the tlc sample with the

30 addition of MeOH. Upon completion of the reaction, as judged by tlc, MeOH (50 mL) was added and the mixture evaporated at 35°C. The residue was dissolved in CHCl₃ (800 mL) and extracted with 2x200 mL of saturated sodium bicarbonate and 2x200 mL of saturated NaCl. The water

35 layers were back extracted with 200 mL of CHCl₃. The

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combined organics were dried with sodium sulfate and evaporated to give 122 g of residue (approx. 90% product). The residue was purified on a 3.5 kg silica gel column and eluted using EtOAc/Hexane (4:1). Pure product fractions
5 were evaporated to yield 96 g (84%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine:

A first solution was prepared by dissolving 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH₃CN (700 mL) and set aside. Triethylamine (189 mL, 1.44 M) was added to a solution of triazole (90 g, 1.3 M) in CH₃CN (1 L), cooled to -5°C and stirred for 0.5 hours using an overhead stirrer. POCl₃ was added dropwise, over a 30 minute period, to the
10 stirred solution maintained at 0-10°C, and the resulting mixture stirred for an additional 2 hours. The first solution was added dropwise, over a 45 minute period, to the later solution. The resulting reaction mixture was stored overnight in a cold room. Salts were filtered from
15 the reaction mixture and the solution was evaporated. The residue was dissolved in EtOAc (1 L) and the insoluble solids were removed by filtration. The filtrate was washed with 1x300 mL of NaHCO₃, and 2x300 mL of saturated NaCl, dried over sodium sulfate and evaporated. The residue was
20 triturated with EtOAc to give the title compound.

25

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine:

A solution of 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine (103 g, 0.141 M) in dioxane (500 mL) and NH₄OH (30 mL) was stirred at room
30 temperature for 2 hours. The dioxane solution was evaporated and the residue azeotroped with MeOH (2x200 mL). The residue was dissolved in MeOH (300 mL) and transferred to a 2 liter stainless steel pressure vessel. MeOH (400 mL) saturated with NH₃ gas was added and the vessel heated
35 to 100°C for 2 hours (tlc showed complete conversion). The

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vessel contents were evaporated to dryness and the residue was dissolved in EtOAc (500 mL) and washed once with saturated NaCl (200 mL). The organics were dried over sodium sulfate and the solvent was evaporated to give 85 g (95%) of the title compound.

5
N⁴-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-
cytidine:

10 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyl-cytidine (85 g, 0.134 M) was dissolved in DMF (800 mL) and benzoic anhydride (37.2 g, 0.165 M) was added with stirring. After stirring for 3 hours, tlc showed the reaction to be approximately 95% complete. The solvent was evaporated and the residue azeotroped with MeOH (200 mL). The residue was dissolved in CHCl₃ (700 mL) and extracted 15 with saturated NaHCO₃ (2x300 mL) and saturated NaCl (2x300 mL), dried over MgSO₄ and evaporated to give a residue (96 g). The residue was chromatographed on a 1.5 kg silica column using EtOAc/Hexane (1:1) containing 0.5% Et₃NH as the eluting solvent. The pure product fractions were 20 evaporated to give 90 g (90%) of the title compound.

N⁴-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-
methylcytidine-3'-amidite:

25 N⁴-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (74 g, 0.10 M) was dissolved in CH₂Cl₂ (1 L). Tetrazole diisopropylamine (7.1 g) and 2-cyanoethoxy-tetra(isopropyl)phosphite (40.5 mL, 0.123 M) were added with stirring, under a nitrogen atmosphere. The resulting mixture was stirred for 20 hours at room temperature (tlc showed the reaction to be 95% complete). The reaction 30 mixture was extracted with saturated NaHCO₃ (1x300 mL) and saturated NaCl (3x300 mL). The aqueous washes were back-extracted with CH₂Cl₂ (300 mL), and the extracts were combined, dried over MgSO₄ and concentrated. The residue obtained was chromatographed on a 1.5 kg silica column 35 using EtOAc\Hexane (3:1) as the eluting solvent. The pure

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fractions were combined to give 90.6 g (87%) of the title compound.

5-methyl-2'-deoxycytidine (5-me-C) containing oligonucleotides were synthesized according to published methods (Sanghvi et al., *Nucl. Acids Res.* 1993, 21, 3197-3203) using commercially available phosphoramidites (Glen Research, Sterling VA or ChemGenes, Needham MA).

Oligonucleotides having methylene(methylimino) (MMI) backbones were synthesized according to U.S. Patent 10 5,378,825, which is coassigned to the assignee of the present invention and is incorporated herein in its entirety. For ease of synthesis, various nucleoside dimers containing MMI linkages were synthesized and incorporated into oligonucleotides. Other nitrogen-containing backbones 15 are synthesized according to WO 92/20823 which is also coassigned to the assignee of the present invention and incorporated herein in its entirety.

Oligonucleotides having amide backbones are synthesized according to De Mesmaeker et al. (*Acc. Chem. 20 Res.* 1995, 28, 366-374). The amide moiety is readily accessible by simple and well-known synthetic methods and is compatible with the conditions required for solid phase synthesis of oligonucleotides.

25 Oligonucleotides with morpholino backbones are synthesized according to U.S. Patent 5,034,506 (Summerton and Weller).

Peptide-nucleic acid (PNA) oligomers are synthesized according to P.E. Nielsen et al. (*Science* 1991, 254, 1497-1500).

30 After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the oligonucleotides are purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Synthesized 35 oligonucleotides were analyzed by polyacrylamide gel

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electrophoresis on denaturing gels and judged to be at least 85% full length material. The relative amounts of phosphorothioate and phosphodiester linkages obtained in synthesis were periodically checked by ^{31}P nuclear magnetic resonance spectroscopy, and for some studies oligonucleotides were purified by HPLC, as described by Chiang et al. (*J. Biol. Chem.* 1991, 266, 18162). Results obtained with HPLC-purified material were similar to those obtained with non-HPLC purified material.

10 **EXAMPLE 2: Human TNF- α Oligodeoxynucleotide Sequences**

Antisense oligonucleotides were designed to target human TNF- α . Target sequence data are from the TNF- α cDNA sequence published by Nedwin, G.E. et al. (*Nucleic Acids Res.* 1985, 13, 6361-6373); Genbank accession number X02910, provided herein as SEQ ID NO: 1. Oligodeoxynucleotides were synthesized primarily with phosphorothioate linkages. Oligonucleotide sequences are shown in Table 1. Oligonucleotide 14640 (SEQ ID NO. 2) is a published TNF- α antisense oligodeoxynucleotide targeted to the start site of the TNF- α gene (Hartmann, G., et al., *Antisense Nucleic Acid Drug Dev.*, 1996, 6, 291-299). Oligonucleotide 2302 (SEQ ID NO. 41) is an antisense oligodeoxynucleotide targeted to the human intracellular adhesion molecule-1 (ICAM-1) and was used as an unrelated (negative) target control. Oligonucleotide 13664 (SEQ ID NO. 42) is an antisense oligodeoxynucleotide targeted to the Herpes Simplex Virus type 1 and was used as an unrelated target control.

30 NeoHK cells, human neonatal foreskin keratinocytes (obtained from Cascade Biologicals, Inc., Portland, OR) were cultured in Keratinocyte medium containing the supplied growth factors (Life Technologies, Rockville, MD).

At assay time, the cells were between 70% and 90% confluent. The cells were incubated in the presence of

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Keratinocyte medium, without the supplied growth factors added, and the oligonucleotide formulated in LIPOFECTIN® (Life Technologies), a 1:1 (w/w) liposome formulation of the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-n,n,n-trimethylammonium chloride (DOTMA), and dioleoyl phosphatidylethanolamine (DOPE) in membrane filtered water. For an initial screen, the oligonucleotide concentration was 300 nM in 9 µg/mL LIPOFECTIN®. Treatment was for four hours. After treatment, the medium was removed and the cells were further incubated in Keratinocyte medium containing the supplied growth factors and 100 nM phorbol 12-myristate 13-acetate (PMA, Sigma, St. Louis, MO). mRNA was analyzed 2 hours post-induction with PMA. Protein levels were analyzed 12 to 20 hours post-induction.

Total mRNA was isolated using the RNEASY® Mini Kit (Qiagen, Valencia, CA; similar kits from other manufacturers may also be used), separated on a 1% agarose gel, transferred to HYBOND™-N+ membrane (Amersham Pharmacia Biotech, Piscataway, NJ), a positively charged nylon membrane, and probed. A TNF- α probe consisted of the 505 bp EcoRI-HindIII fragment from BBG 18 (R&D Systems, Minneapolis, MN), a plasmid containing human TNF- α cDNA. A glyceraldehyde 3-phosphate dehydrogenase (G3PDH) probe consisted of the 1.06 kb HindIII fragment from pHC GAP (American Type Culture Collection, Manassas, VA), a plasmid containing human G3PDH cDNA. The restriction fragments were purified from low-melting temperature agarose, as described in Maniatis, T., et al., *Molecular Cloning: A Laboratory Manual, 1989* and labeled with REDIVUE™ 32 P-dCTP (Amersham Pharmacia Biotech, Piscataway, NJ) and PRIME-A-GENE® labeling kit (Promega, Madison, WI). mRNA was quantitated by a PhosphoImager (Molecular Dynamics, Sunnyvale, CA).

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Secreted TNF- α protein levels were measured using a human TNF- α ELISA kit (R&D Systems, Minneapolis, MN or Genzyme, Cambridge, MA).

TABLE 1

5 Nucleotide Sequences of Human TNF- α Phosphorothioate Oligodeoxynucleotides

	ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
10	14640	CATG <u>CTTCAGTGCTCAT</u>	2	0796-0813	AUG
	14641	TGAGGGAG <u>CGTCTGCTGGCT</u>	3	0615-0634	5'-UTR
	14642	GTG <u>CTCATGGTGTCTTTCC</u>	4	0784-0803	AUG
	14643	TAAT <u>CACAAGTGCAAACATA</u>	5	3038-3057	3'-UTR
	14644	TAC <u>CCCCGGTCTCCAAATAA</u>	6	3101-3120	3'-UTR
15	14810	GTG <u>CTCATGGTGTCCCTTCC</u>	4	0784-0803	AUG
	14811	AGCACCGC <u>CTGGAGGCCCT</u>	7	0869-0886	coding
	14812	GCTGAGGAAC <u>AAAGCACCGCC</u>	8	0878-0897	coding
	14813	AGGCAGAA <u>AGAGCGTGGTGGC</u>	9	0925-0944	coding
	14814	AAAGTGCAG <u>CAGGCAGAAGA</u>	10	0935-0954	coding
20	14815	TTAGAGAG <u>AGGGTCCCTGG</u>	11	1593-1610	coding
	14816	TGACTGC <u>CTGGGCCAGAG</u>	12	1617-1634	junction
	14817	GGGTC <u>GAGAAAGATGATC</u>	13	1822-1839	junction
	14818	GGGCTACAG <u>GGCTTGTCACTC</u>	14	1841-1860	coding
	14820	CCC <u>CTCAGGTTGAGGGTTG</u>	15	2171-2190	junction
25	14821	CCATTGG <u>CCAGGAGGGCATT</u>	16	2218-2237	coding
	14822	ACCACCA <u>GCTGGTTATCTCT</u>	17	2248-2267	coding
	14823	CTGGGAG <u>TAGATGAGGTACA</u>	18	2282-2301	coding
	14824	CC <u>CTTGAAGAGGACCTGGGA</u>	19	2296-2315	coding
	14825	GGTGTGG <u>GTGAGGAGCACAT</u>	20	2336-2355	coding
30	14826	GT <u>CTGGTAGGAGACGGCGAT</u>	21	2365-2384	coding
	14827	GCAGAGAG <u>GGAGGTTGACCTT</u>	22	2386-2405	coding
	14828	GCTTGG <u>CCTCAGCCCCCTCT</u>	23	2436-2455	coding

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14829	CCTCCCAGATAGATGGGCTC	24	2464-2483	coding
14830	CCCTTCTCCAGCTGGAAGAC	25	2485-2504	coding
14831	ATCTCAGCGCTGAGTCGGTC	26	2506-2525	coding
14832	TCGAGATAAGTCGGGCCGATT	27	2527-2546	coding
5	14833 AAGTAGACCTGCCAGACTC	28	2554-2573	coding
	14834 GGATGTTCGTCCTCCTCACCA	29	2588-2607	STOP
	14835 ACCCTAACCCCCAATTCTC	30	2689-2708	3'-UTR
	14836 CCACACATTCTGAATCCCCA	31	2758-2777	3'-UTR
	14837 AGGCCCCAGTGAGTTCTGGA	32	2825-2844	3'-UTR
10	14838 GTCTCCAGATTCCAGATGTC	33	2860-2879	3'-UTR
	14839 CTCAAGTCCTGCAGCATTCT	34	2902-2921	3'-UTR
	14840 TGGGTCCCCCAGGATAACCCC	35	3115-3134	3'-UTR
	14841 ACGGAAAACATGTCTGAGCC	36	3151-3170	3'-UTR
	14842 CTCCGTTTCACGGAAAACA	37	3161-3180	3'-UTR
15	14843 GCCTATTGTTCAGCTCCGTT	38	3174-3193	3'-UTR
	14844 GGTCAACCAAATCAGCATTGT	39	3272-3292	3'-UTR
	14845 GAGGCTCAGCAATGAGTGAC	40	3297-3316	3'-UTR
	2302 <u>GCCCAAGCTGGCATCCGTCA</u>	41		target control
20	13664 GCCGAGGTCCATGTCGTACGC	42		target control

¹ "C" residues are 5-methyl-cytosines except "C" residues are unmodified cytidines; all linkages are phosphorothioate linkages.

²Co-ordinates from Genbank Accession No. X02910, locus name "HSTNFA", SEQ ID NO. 1.

Results are shown in Table 2. Oligonucleotides 14828 (SEQ ID NO. 23), 14829 (SEQ ID NO. 24), 14832 (SEQ ID NO. 27), 14833 (SEQ ID NO. 28), 14834 (SEQ ID NO. 29), 14835 (SEQ ID NO. 30), 14836 (SEQ ID NO. 31), 14839 (SEQ ID NO. 34), 14840 (SEQ ID NO. 35), and 14844 (SEQ ID NO. 39) inhibited TNF- α expression by approximately 50% or more.

35 Oligonucleotides 14828 (SEQ ID NO. 23), 14834 (SEQ ID NO.

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29), and 14840 (SEQ ID NO. 35) gave better than 70% inhibition.

TABLE 2

Inhibition of Human TNF- α mRNA Expression by
5 Phosphorothioate Oligodeoxynucleotides

	ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
	basal	---	---	16%	---
	induced	---	---	100%	0%
10	13664	42	control	140%	---
	14640	2	AUG	61%	39%
	14641	3	5' -UTR	95%	5%
	14642	4	AUG	131%	---
	14810	4	AUG	111%	---
15	14815	11	coding	85%	15%
	14816	12	junction	106%	---
	14817	13	junction	97%	3%
	14818	14	coding	64%	36%
	14820	15	junction	111%	---
20	14821	16	coding	91%	9%
	14822	17	coding	57%	43%
	14827	22	coding	67%	33%
	14828	23	coding	27%	73%
	14829	24	coding	33%	67%
25	14830	25	coding	71%	29%
	14831	26	coding	62%	38%
	14832	27	coding	40%	60%
	14833	28	coding	43%	57%
	14834	29	STOP	26%	74%
30	14835	30	3' -UTR	32%	68%
	14836	31	3' -UTR	40%	60%

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	14837	32	3'-UTR	106%	---
	14838	33	3'-UTR	70%	30%
	14839	34	5'-UTR	49%	51%
	14840	35	3'-UTR	28%	72%
5	14841	36	3'-UTR	60%	40%
	14842	37	3'-UTR	164%	---
	14843	38	3'-UTR	67%	33%
	14844	39	3'-UTR	46%	54%
	14845	40	3'-UTR	65%	35%

10 **EXAMPLE 3: Dose response of antisense phosphorothioate
oligodeoxynucleotide effects on human TNF- α mRNA levels in
NeoHK cells**

Four of the more active oligonucleotides from the initial screen were chosen for dose response assays. These include 15 oligonucleotides 14828 (SEQ ID NO. 23), 14833 (SEQ ID NO. 28), 14834 (SEQ ID NO. 29) and 14839 (SEQ ID NO. 34). NeoHK cells were grown, treated and processed as described in Example 2. LIPOFECTIN® was added at a ratio of 3 μ g/mL per 100 nM of oligonucleotide. The control included LIPOFECTIN® at a 20 concentration of 9 μ g/mL. The effect of the TNF- α antisense oligonucleotides was normalized to the non-specific target control. Results are shown in Table 3. Each oligonucleotide showed a dose response effect with maximal inhibition greater than 70%. Oligonucleotides 14828 (SEQ ID NO. 23) had an IC₅₀ 25 of approximately 185 nM. Oligonucleotides 14833 (SEQ ID NO. 28) had an IC₅₀ of approximately 150 nM. Oligonucleotides 14834 (SEQ ID NO. 29) and 14839 (SEQ ID NO. 34) had an IC₅₀ of approximately 140 nM.

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TABLE 3

Dose Response of NeoHK Cells to TNF- α

Antisense Phosphorothioate Oligodeoxynucleotides (ASOs)

ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% mRNA Expression	% mRNA Inhibition
5	2302	41 control	25 nM	100%	---
	"	"	50 nM	100%	---
	"	"	100 nM	100%	---
	"	"	200 nM	100%	---
	"	"	300 nM	100%	---
10	14828	23 coding	25 nM	122%	---
	"	"	50 nM	97%	3%
	"	"	100 nM	96%	4%
	"	"	200 nM	40%	60%
	"	"	300 nM	22%	78%
15	14833	28 coding	25 nM	89%	11%
	"	"	50 nM	78%	22%
	"	"	100 nM	64%	36%
	"	"	200 nM	36%	64%
	"	"	300 nM	25%	75%
20	14834	29 STOP	25 nM	94%	6%
	"	"	50 nM	69%	31%
	"	"	100 nM	65%	35%
	"	"	200 nM	26%	74%
	"	"	300 nM	11%	89%
25	14839	34 3'-UTR	25 nM	140%	---
	"	"	50 nM	112%	---
	"	"	100 nM	65%	35%
	"	"	200 nM	29%	71%
	"	"	300 nM	22%	78%

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EXAMPLE 4: Design and Testing of Chimeric (deoxy gapped) 2'-O-methoxyethyl TNF- α Antisense Oligonucleotides on TNF- α Levels in NeoHK Cells

Oligonucleotides having SEQ ID NO: 28 and SEQ ID NO: 29
5 were synthesized as uniformly phosphorothioate or mixed phosphorothioate/phosphodiester chimeric oligonucleotides having variable regions of 2'-O-methoxyethyl (2'-MOE) nucleotides and deoxynucleotides. The sequences and the oligonucleotide chemistries are shown in Table 4. All 2'-MOE
10 cytosines were 5-methyl-cytosines.

Dose response experiments, as discussed in Example 3, were performed using these chimeric oligonucleotides. The effect of the TNF- α antisense oligonucleotides was normalized to the non-specific target control. Results are shown in
15 Table 5. The activities of the chimeric oligonucleotides tested were comparable to the parent phosphorothioate oligonucleotide.

TABLE 4

Nucleotide Sequences of TNF- α Chimeric (deoxy gapped) 2'-O-methoxyethyl Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE (5' -> 3')	SEQ ID NO:	TARGET NUCLEOTIDE CO-ORDINATES ¹	GENE TARGET REGION
5	AsASGSTsAsGSAsCSCsTsGSCsCsAsGsAsCstsC	28	2554-2573	coding
16467	AoAOGotoAsGSAsCSCsTsGSCsCsAsGoAOCoToC	28	2554-2573	coding
16468	AsASGSTsAsGSAsCSCsTsGSCsCsAsGsAsCstsC	28	2554-2573	coding
16469	AsASGSTsAsGSAsCSCsTsGSCsCsAsGsAsCstsC	28	2554-2573	coding
16470	AsASGSTsAsGSAsCSCsTsGSCsCsAsGsAsCstsC	28	2554-2573	coding
10	AsASGSTsAsGSAsCSCsTsGSCsCsAsGsAsCstsC	28	2554-2573	coding
14834	GSGsASTsGSTsTsCGsTsCsCstsCsAsCsA	29	2588-2607	STOP
16472	GoGOAtoGStsTsCGsTsCsCstsCsCsToCoAOCoA	29	2588-2607	STOP
16473	GSGsASTsGSTsTsCGsTsCsCstsCsAsCsA	29	2588-2607	STOP
16474	GSGsASTsGSTsTsCsGstsCsCstsCsAsCsA	29	2588-2607	STOP
15	GSGsAsTsGstsTsCsGstsCsCsTsCsAsCsA	29	2588-2607	STOP
16476	GSGsASTsGSTsTsCGsTsCsCstsCsAsCsA	29	2588-2607	STOP

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-) . All 2'-methoxyethoxy cytidines are 5-methyl-cytidines; "s" linkages are phosphorothioate linkages, "o" linkages are phosphodiester linkages.

² Co-ordinates from Genbank Accession No. X02910, locus name "HSTMFA", SEQ ID NO. 1.

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TABLE 5

Dose Response of NeoHK Cells to TNF- α
 Chimeric (deoxy gapped) 2'-O-methoxyethyl Antisense
 Oligonucleotides

		SEQ ID NO:	ASO Gene Target	Dose	% mRNA Expression	% mRNA Inhibition
5	13664	42	control	50 nM	100%	---
		"	"	100 nM	100%	---
		"	"	200 nM	100%	---
		"	"	300 nM	100%	---
10	14833	28	coding	50 nM	69%	31%
		"	"	100 nM	64%	36%
		"	"	200 nM	56%	44%
		"	"	300 nM	36%	64%
		16468	coding	50 nM	66%	34%
15	"	"	"	100 nM	53%	47%
		"	"	200 nM	34%	66%
		"	"	300 nM	25%	75%
20	16471	28	coding	50 nM	77%	23%
		"	"	100 nM	56%	44%
		"	"	200 nM	53%	47%
25	"	"	"	300 nM	31%	69%
		14834	STOP	50 nM	74%	26%
		"	"	100 nM	53%	47%
30	16473	29	STOP	200 nM	24%	76%
		"	"	300 nM	11%	89%
		16473	29	STOP	50 nM	71%
		"	"	100 nM	51%	49%
30	16476	29	STOP	200 nM	28%	72%
		"	"	300 nM	23%	77%
		16476	29	STOP	50 nM	74%
						26%

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"	"	"	100 nM	58%	42%
"	"	"	200 nM	32%	68%
"	"	"	300 nM	31%	69%

EXAMPLE 5: Design and Testing of Chimeric

5 **Phosphorothioate/MMI TNF- α Antisense Oligodeoxynucleotides
on TNF- α Levels in NeoHK Cells**

Oligonucleotides having SEQ ID NO. 29 were synthesized as mixed phosphorothioate/methylene(methylimino) (MMI) chimeric oligodeoxynucleotides. The sequences and the 10 oligonucleotide chemistries are shown in Table 6.

Oligonucleotide 13393 (SEQ ID NO. 49) is an antisense oligonucleotide targeted to the human intracellular adhesion molecule-1 (ICAM-1) and was used as an unrelated target control. All cytosines were 5-methyl-cytosines.

15 Dose response experiments were performed using these chimeric oligonucleotides, as discussed in Example 3 except quantitation of TNF- α mRNA levels was determined by real-time PCR (RT-PCR) using the ABI PRISM™ 7700 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) 20 according to manufacturer's instructions. This is a closed-tube, non-gel-based, fluorescence detection system which allows high-throughput quantitation of polymerase chain reaction (PCR) products in real-time. As opposed to standard PCR, in which amplification products are 25 quantitated after the PCR is completed, products in RT-PCR are quantitated as they accumulate. This is accomplished by including in the PCR reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent dyes. A reporter 30 dye (e.g., JOE or FAM, PE-Applied Biosystems, Foster City, CA) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, PE-Applied Biosystems, Foster City, CA) is

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attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a
5 substrate that can be cleaved by the 5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a
10 sequence-specific fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the fluorescence intensity is monitored at regular (six-second) intervals by laser optics built into the ABI PRISM™ 7700 Sequence
15 Detection System. In each assay, a series of parallel reactions containing serial dilutions of mRNA from untreated control samples generates a standard curve that is used to quantitate the percent inhibition after antisense oligonucleotide treatment of test samples.

20 RT-PCR reagents were obtained from PE-Applied Biosystems, Foster City, CA. RT-PCR reactions were carried out by adding 25 µl PCR cocktail (1x TAQMAN® buffer A, 5.5 mM MgCl₂, 300 µM each of dATP, dCTP and dGTP, 600 µM of dUTP, 100 nM each of forward primer, reverse primer, and
25 probe, 20 U RNase inhibitor, 1.25 units AMPLITAQ GOLD®, and 12.5 U MuLV reverse transcriptase) to 96 well plates containing 25 µl poly(A) mRNA solution. The RT reaction was carried out by incubation for 30 minutes at 48°C. following a 10 minute incubation at 95°C to activate the AMPLITAQ
30 GOLD®, 40 cycles of a two-step PCR protocol were carried out: 95°C for 15 seconds (denaturation) followed by 60°C for 1.5 minutes (annealing/extension).

For TNF-α the PCR primers were:

Forward: 5'-CAGGCGGTGCTTGTTCCT-3' SEQ ID NO. 43

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Reverse: 5'-GCCAGAGGGCTGATTAGAGAGA-3' SEQ ID NO. 44 and the PCR probe was: FAM-CTTCTCCTCCTGATCGTGGCAGGC-TAMRA (SEQ ID NO. 45) where FAM or JOE (PE-Applied Biosystems, Foster City, CA) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, CA) is the quencher dye.

For GAPDH the PCR primers were:

Forward primer: 5'-GAAGGTGAAGGTCGGAGTC-3' SEQ ID NO. 46

Reverse primer: 5'-GAAGATGGTGATGGGATTTC-3' SEQ ID NO. 47

10 and the PCR probe was: 5' JOE-CAAGCTTCCCGTTCTCAGCC - TAMRA 3' (SEQ ID NO. 48) where FAM or JOE (PE-Applied Biosystems, Foster City, CA) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, CA) is the quencher dye.

15 Results are shown in Table 7. The oligonucleotide containing MMI linkages was more effective in reducing TNF- α mRNA levels than the uniformly phosphorothioate oligonucleotide. The IC₅₀ value was reduced from approximately 75 nM, for oligonucleotide 14834 (SEQ ID NO: 29), to approximately 30 nM for oligonucleotide 16922 (SEQ ID NO: 29).

Dose response experiments were also performed measuring the effect on TNF- α protein levels. Protein levels were measured as described in Example 2. Results 25 are shown in Table 8. The oligonucleotide containing four MMI linkages on each end was more effective in reducing protein levels than the uniformly phosphorothioate oligonucleotide. The IC₅₀ value was reduced from approximately 90 nM, for oligonucleotide 14834 (SEQ ID NO: 29), to approximately 45 nM for oligonucleotide 16922 (SEQ ID NO: 29).

TABLE 6

Nucleotide Sequences of Human TNF- α Chimeric Phosphorothioate/MMI Oligodeoxynucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE (5' -> 3')	SEQ NO:	TARGET ID NUCLEOTIDE CO-ORDINATES ¹	GENE TARGET REGION
5 14834	GsGsAsTsGstTsTsCsGsTsCsCstTsCsCsTscsAsCsA	29	2588-2607	STOP
16922	GmGmAmTmGstTsCsGsTsCstScsCstTsCsTmCmAmCmA	29	2588-2607	STOP
16923	GmGmAmTmGmTmTsCsGsTsCsCstScsCmCmTmCmAmCmA	29	2588-2607	STOP
13393	TsCstSgsAsGstsAsGstsAsGsAsGsCsTsc	49	target control	

¹ All cytosine residues are 5-methyl-cytosines; "s" linkages are phosphorothioate linkages, "m" linkages are methylene(methylimino) (MMI).

² Co-ordinates from Genbank Accession No. X02910, locus name "HSTMFA", SEQ ID NO. 1.

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TABLE 7

Dose Response of Chimeric Phosphorothioate/MMI TNF- α
 Antisense Oligodeoxynucleotides on TNF- α mRNA Levels in
 PMA-Induced NeoHK Cells

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% mRNA Expression	% mRNA Inhibition
5	induced	---	---	---	100%	---
	13393	49	control	25 nM	87.3%	12.7%
	"	"	"	50 nM	98.5%	1.5%
	"	"	"	100 nM	133.1%	---
10	"	"	"	200 nM	139.6%	---
	14834	29	STOP	25 nM	98.7%	1.3%
	"	"	"	50 nM	70.8%	29.2%
	"	"	"	100 nM	36.0%	64.0%
	"	"	"	200 nM	38.2%	61.8%
15	16922	29	STOP	25 nM	58.9%	41.1%
	"	"	"	50 nM	28.2%	71.8%
	"	"	"	100 nM	22.2%	77.8%
	"	"	"	200 nM	18.9%	81.1%

TABLE 8

20 Dose Response of Chimeric Phosphorothioate/MMI TNF- α
 Antisense Oligodeoxynucleotides on TNF- α Protein Levels in
 PMA-Induced NeoHK Cells

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% protein Expression	% protein Inhibition
	induced	---	---	---	100.0%	---
25	13393	49	control	25 nM	117.0%	---
	"	"	"	50 nM	86.6%	13.4%

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	"	"	"	100 nM	98.7%	1.3%
	"	"	"	200 nM	78.0%	22.0%
14834	29	STOP		25 nM	84.8%	15.2%
	"	"	"	50 nM	76.9%	23.1%
5	"	"	"	100 nM	44.5%	55.5%
	"	"	"	200 nM	18.7%	81.3%
16922	29	STOP		25 nM	67.1%	32.9%
	"	"	"	50 nM	48.6%	51.4%
	"	"	"	100 nM	20.0%	80.0%
10	"	"	"	200 nM	7.9%	92.1%
16923	29	STOP		25 nM	79.9%	20.1%
	"	"	"	50 nM	69.9%	30.1%
	"	"	"	100 nM	56.0%	44.0%
	"	"	"	200 nM	44.5%	55.5%

15 EXAMPLE 6: Additional Human TNF- α Antisense Oligonucleotide Sequences

A second screening of human TNF- α antisense oligonucleotides was performed. Oligonucleotides were designed specifically against specific regions of the TNF- α gene. A series of oligonucleotides was designed to target introns 1 and 3, and exon 4. Sequences targeting introns 1 or 3 were synthesized as uniformly phosphorothioate oligodeoxynucleotides or mixed phosphorothioate/phosphodiester chimeric backbone oligonucleotides having variable regions of 2'-O-methoxyethyl (2'-MOE) nucleotides and deoxynucleotides. Sequences targeting exon 4 were synthesized as mixed phosphorothioate/phosphodiester chimeric backbone oligonucleotides having variable regions of 2'-O-methoxyethyl (2'-MOE) nucleotides and deoxynucleotides. The sequences of the chimeric oligonucleotides are shown in Table 9. Sequences of the

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uniformly phosphorothioate oligodeoxynucleotides are shown in Table 11.

These oligonucleotides were screened at 50 nM and 200 nM for their ability to inhibit TNF- α protein secretion, 5 essentially as described in Example 2. Results for the chimeric backbone oligonucleotides are shown in Table 10; results for the uniformly phosphorothioate oligodeoxynucleotides are shown in Table 12.

For the chimeric backbone oligonucleotides targeting 10 introns 1 or 3, oligonucleotide 21688 (SEQ ID NO. 69) gave 60% inhibition or greater. For chimeric backbone oligonucleotides targeting exon 4, two-thirds of the oligonucleotides gave nearly 60% inhibition or greater (SEQ ID NOS. 88, 90, 91, 92, 93, 94, 97, and 98). See Table 10. 15 For the uniformly phosphorothioate oligodeoxynucleotides, five of nine oligonucleotides targeting intron 3 were effective in reducing TNF- α expression by nearly 60% or greater (SEQ ID NOS. 79, 80, 81, 82, and 84). See Table 12.

20 Oligonucleotides having SEQ ID NO. 91 and SEQ ID NO. 98 were synthesized as a uniformly phosphorothioate oligodeoxynucleotides or mixed phosphorothioate/phosphodiester chimeric backbone oligonucleotides having variable regions of 2'-O-methoxyethyl (2'-MOE) nucleotides 25 and deoxynucleotides. The sequences and the oligonucleotide chemistries are shown in Table 13. All 2'-MOE cytosines and 2'-deoxy cytosines were 5-methyl-cytosines.

Dose response experiments, as discussed in Example 3, 30 were performed using these oligonucleotides. Included in this experiment were two oligonucleotides targeting intron 1 and two oligonucleotides targeting intron 3. Results are shown in Tables 14 and 15. The oligonucleotides targeting exon 4 with variable regions of 2'-O-methoxyethyl (2'-MOE)

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nucleotides and deoxynucleotides and/or uniformly phosphorothioate or mixed phosphorothioate/phosphodiester were, in general, comparable to the parent compound.

Oligonucleotides targeting introns 1 or 3 having SEQ 5 ID NOS 66, 69 and 80 were effective in reducing TNF- α mRNA levels by greater than 80% and showed a dose response effect with an IC₅₀ approximately 110 nM. See Tables 14 and 15.

TABLE 9
Nucleotide Sequences of TNF- α Chimeric Backbone (deoxy gapped) 2'-O-methoxyethyl Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE (5' -> 3')	SEQ ID NO:		TARGET GENE NUCLEOTIDE CO-ORDINATES ¹	GENE TARGET REGION
		56	-		
21669	T o G o C o G o T o C o S t C o A ST S t C o S t C o T o C o T o T	50		1019-1038	intron 1
21670	T o C o C o A ST S t C o S t C o T o C o T o C o T	51		1039-1058	intron 1
21671	C o A o G o C o G s C o S As S t C o S t S t S o C o C o C o A	52		1059-1078	intron 1
21672	T o C o T o C o T o T o C o S t C o S t C o S As S t C o S t C o T o A o T	53		1079-1098	intron 1
10	C o G o T o C o T o T o C o S t C o S t C o S As S t G s T o T o T o T o T	54		1099-1118	intron 1
21674	C o A o C o A o T o C o S t C o S t C o S As S t G s C s A o T o C o C o C	55		1119-1138	intron 1
21675	C o T o C o T o C o T o C o S t C o S t C o S As S t C o T o T o G o C	56		1139-1158	intron 1
21676	G o T o C o T o C o S t C o S As S t C o S t T o T o C o T	57		1159-1178	intron 1
21677	T o T o C o A o T o C o S t G s C o S As G s A o T o C o C	58		1179-1198	intron 1
15	A o T o A o C o A o S As C o T o T o S As G s T o G s A o C o A o C o C	59		1199-1218	intron 1
21679	T o T o C o A o T o C o S t G s C o S As S t T o C o T o C o C	60		1219-1238	intron 1
21680	T o A o T o A o T o C o S t G s C o S As S t G s T o T o C o A	61		1239-1258	intron 1
21681	C o T o G o T o C o S t C o S As S t A o T o T o T o A	62		1259-1278	intron 1

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21700	CAGGTTTGCAGGTAAATGCCAAGCCTGAA	81	2011-2030	intron 3	
21701	CCAACTTCCCTTGGGCGGAACTTAA	82	2031-2050	intron 3	
21702	GTTTCCCTTGGTCTGCTGCTGCTGAA	83	2051-2070	intron 3	
21703	CCACCCCTTGGGCTGCTGCTGCTGAA	84	2071-2090	intron 3	
5	TGTTCCCTTGGGCTGCTGCTGCTGAA	85	2091-2110	intron 3	
21704	AATTTGGGCTGCTGCTGCTGCTGAA	86	2111-2130	intron 3	
21705	AACTTGGGCTGCTGCTGCTGCTGAA	87	2131-2150	intron 3	
21706	AAAGGAAAGGAAAGGAAAGGAAAGGAA	88	2561-2580	exon 4	
21722	GAATTCCTTGGGCTGCTGCTGCTGAA	89	2541-2560	exon 4	
21723	CAAGGAACTTGGGCTGCTGCTGCTGAA	90	2521-2540	exon 4	
10	21724	TAATGTTGGGCTGCTGCTGCTGAA	91	2501-2520	exon 4
21725	AGGCTTGGGCTGCTGCTGCTGCTGAA	92	2481-2500	exon 4	
21726	TCTTGGGCTTGGGCTGCTGCTGCTGAA	93	2461-2480	exon 4	
21727	CCCTTGGGCTTGGGCTGCTGCTGCTGAA	94	2441-2460	exon 4	
21728	CCCTTGGGCTTGGGCTGCTGCTGCTGAA	95	2421-2440	exon 4	
15	21729	CCCTTGGGCTTGGGCTGCTGCTGCTGAA	96	2401-2420	exon 4
21730	CCCTTGGGCTTGGGCTGCTGCTGCTGAA				

21731	G AAoGooAsGSGsTtsTsGAsCSCTsTsGotoCoT	97	2381-2400	exon 4
21732	G oGotoAoSgsAsGsAsCsGSGsCSGsASToGoCoG	98	2361-2380	exon 4
21733	C oToGoAotTsGSGsTsGstTsGsgsGstsGsAoGoGoAOG	99	2341-2360	exon 4

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethoxy cytidines and 2'-deoxycytidines are 5-methyl-cytidines; "s" linkages are phosphorothioate linkages, "o" linkages are phosphodiester linkages.

² Coordinates from Genbank Accession No. X02910, locus name "HSTNFA", SEQ ID NO. 1.

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TABLE 10

Dose Response of PMA-Induced neoHK Cells to Chimeric
 Backbone (deoxy gapped) 2'-O-methoxyethyl TNF- α Antisense
 Oligonucleotides

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% protein Expression	% protein Inhibition
	induced	---	---	---	100%	---
5	14834	29	STOP	50 nM	76%	24%
	"	"	"	200 nM	16%	84%
	21669	50	intron 1	50 nM	134%	---
10	"	"	"	200 nM	114%	---
	21670	51	intron 1	50 nM	122%	---
	"	"	"	200 nM	101%	---
	21671	52	intron 1	50 nM	90%	10%
	"	"	"	200 nM	58%	42%
15	21672	53	intron 1	50 nM	122%	---
	"	"	"	200 nM	131%	---
	21673	54	intron 1	50 nM	102%	---
	"	"	"	200 nM	110%	---
	21674	55	intron 1	50 nM	111%	---
20	"	"	"	200 nM	96%	4%
	21675	56	intron 1	50 nM	114%	---
	"	"	"	200 nM	99%	1%
	21676	57	intron 1	50 nM	107%	---
	"	"	"	200 nM	96%	4%
25	21677	58	intron 1	50 nM	86%	14%
	"	"	"	200 nM	95%	5%
	21678	59	intron 1	50 nM	106%	---
	"	"	"	200 nM	107%	---
	21679	60	intron 1	50 nM	75%	25%
30	"	"	"	200 nM	73%	27%

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	21680	61	intron 1	50 nM	76%	24%
	"	"	"	200 nM	80%	20%
	21681	62	intron 1	50 nM	79%	21%
	"	"	"	200 nM	82%	18%
5	21682	63	intron 1	50 nM	102%	---
	"	"	"	200 nM	88%	12%
	21683	64	intron 1	50 nM	80%	20%
	"	"	"	200 nM	66%	34%
	21684	65	intron 1	50 nM	91%	9%
10	"	"	"	200 nM	69%	31%
	21685	66	intron 1	50 nM	98%	2%
	"	"	"	200 nM	90%	10%
	21686	67	intron 1	50 nM	97%	3%
	"	"	"	200 nM	72%	28%
15	21687	68	intron 1	50 nM	103%	---
	"	"	"	200 nM	64%	36%
	21688	69	intron 1	50 nM	87%	13%
	"	"	"	200 nM	40%	60%
	21689	70	intron 1	50 nM	78%	22%
20	"	"	"	200 nM	74%	26%
	21690	71	intron 1	50 nM	84%	16%
	"	"	"	200 nM	80%	20%
	21691	72	intron 1	50 nM	86%	14%
	"	"	"	200 nM	75%	25%
25	21692	73	intron 1	50 nM	85%	15%
	"	"	"	200 nM	61%	39%
	21693	74	intron 3	50 nM	81%	19%
	"	"	"	200 nM	83%	17%
	21694	75	intron 3	50 nM	99%	1%
30	"	"	"	200 nM	56%	44%
	21695	76	intron 3	50 nM	87%	13%
	"	"	"	200 nM	84%	16%

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	21696	77	intron 3	50 nM	103%	---
	"	"	"	200 nM	86%	14%
	21697	78	intron 3	50 nM	99%	1%
	"	"	"	200 nM	52%	48%
5	21698	79	intron 3	50 nM	96%	4%
	"	"	"	200 nM	47%	53%
	21699	80	intron 3	50 nM	73%	27%
	"	"	"	200 nM	84%	16%
	21700	81	intron 3	50 nM	80%	20%
10	"	"	"	200 nM	53%	47%
	21701	82	intron 3	50 nM	94%	6%
	"	"	"	200 nM	56%	44%
	21702	83	intron 3	50 nM	86%	14%
	"	"	"	200 nM	97%	3%
15	21703	84	intron 3	50 nM	88%	12%
	"	"	"	200 nM	74%	26%
	21704	85	intron 3	50 nM	69%	31%
	"	"	"	200 nM	65%	35%
	21705	86	intron 3	50 nM	92%	8%
20	"	"	"	200 nM	77%	23%
	21706	87	intron 3	50 nM	95%	5%
	"	"	"	200 nM	82%	18%
	21722	88	exon 4	50 nM	81%	19%
	"	"	"	200 nM	41%	59%
25	21723	89	exon 4	50 nM	87%	13%
	"	"	"	200 nM	74%	26%
	21724	90	exon 4	50 nM	68%	32%
	"	"	"	200 nM	33%	67%
	21725	91	exon 4	50 nM	55%	45%
30	"	"	"	200 nM	30%	70%
	21726	92	exon 4	50 nM	72%	28%
	"	"	"	200 nM	40%	60%

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	21727	93	exon 4	50 nM	67%	33%
	"	"	"	200 nM	40%	60%
	21728	94	exon 4	50 nM	62%	38%
	"	"	"	200 nM	41%	59%
5	21729	95	exon 4	50 nM	78%	22%
	"	"	"	200 nM	53%	47%
	21730	96	exon 4	50 nM	68%	32%
	"	"	"	200 nM	48%	52%
	21731	97	exon 4	50 nM	77%	23%
10	"	"	"	200 nM	41%	59%
	21732	98	exon 4	50 nM	62%	38%
	"	"	"	200 nM	28%	72%
	21733	99	exon 4	50 nM	92%	8%
	"	"	"	200 nM	74%	26%

15

TABLE 11

**Nucleotide Sequences of Additional Human TNF- α
Phosphorothioate Oligodeoxynucleotides**

	ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
20	21804	TGCGTCTCTCATTTCCCCTT	50	1019-1038	intron 1
	21805	TCCCCATCTCTCTCCCTCTCT	51	1039-1058	intron 1
	21806	CAGCGCACATCTTCACCCA	52	1059-1078	intron 1
	21807	TCTCTCTCATCCCTCCCTAT	53	1079-1098	intron 1
	21808	CGTCTTCTCCATGTTTTT	54	1099-1118	intron 1
25	21809	CACATCTCTTCTGCATCCC	55	1119-1138	intron 1
	21810	CTCTCTTCCCCATCTCTTG	56	1139-1158	intron 1
	21811	GTCTCTCCATCTTCCTTCT	57	1159-1178	intron 1
	21812	TTCCATGTGCCAGACATCCT	58	1179-1198	intron 1
	21813	ATACACACTTAGTGAGCACC	59	1199-1218	intron 1
30	21814	TTCAATTCAATTCACTCC	60	1219-1238	intron 1

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	21815	TATATCTGCTTGTTCATTCA	61	1239-1258	intron 1
	21816	CTGTCTCCATATCTTATTAA	62	1259-1278	intron 1
	21817	TCTCTTCTCACACCCCCACAT	63	1279-1298	intron 1
	21818	CACTTGTTCTTCCCCCATC	64	1299-1318	intron 1
5	21819	CTCACCATCTTATTACATAT	65	1319-1338	intron 1
	21820	ATATTTCCCGCTCTTCTGT	66	1339-1358	intron 1
	21821	CATCTCTCCTTAGCTGTC	67	1359-1378	intron 1
	21822	TCTTCTCTCCTTATCTCCC	68	1379-1398	intron 1
	21823	GTGTGCCAGACACCCTATCT	69	1399-1418	intron 1
	21824	TCTTCCCTGAGTGTCTTCT	70	1419-1438	intron 1
10	21825	ACCTTCCAGCATTCAACAGC	71	1439-1458	intron 1
	21826	CTCCATTCACTGTGTATTCT	72	1459-1478	intron 1
	21827	TGAGGTGTCTGGTTTCTCT	73	1479-1498	intron 1
	21828	ACACATCCTCAGAGCTCTTA	74	1871-1890	intron 3
	21829	CTAGCCCTCCAAGTCCAAG	75	1891-1910	intron 3
15	21830	CGGGCTTCAATCCCCAAATC	76	1911-1930	intron 3
	21831	AAGTTCTGCCTACCATCAGC	77	1931-1950	intron 3
	21832	GTCCTTCTCACATTGTCTCC	78	1951-1970	intron 3
	21833	CCTTCCCTTGAGCTCAGCGA	79	1971-1990	intron 3
	21834	GGCCTGTGCTGTTCCCTCCAC	80	1991-2010	intron 3
20	21835	CGTTCTGAGTATCCCACTAA	81	2011-2030	intron 3
	21836	CACATCCCACCTGGCCATGA	82	2031-2050	intron 3
	21837	GTCCTCTCTGTCTGTCATCC	83	2051-2070	intron 3
	21838	CCACCCCCACATCCGGTTCCCT	84	2071-2090	intron 3
	21839	TCCTGGCCCTCGAGCTCTGC	85	2091-2110	intron 3
25	21840	ATGTCGGTTCACTCTCCACA	86	2111-2130	intron 3
	21841	AGAGGAGAGTCAGTGTGGCC	87	2131-2150	intron 3

¹ All "C" residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

30 ²Co-ordinates from Genbank Accession No. X02910, locus name "HSTNFA", SEQ ID NO. 1.

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TABLE 12

Dose Response of PMA-Induced neoHK Cells to TNF- α
 Antisense Phosphorothioate Oligodeoxynucleotides

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% protein Expression	% protein Inhibition
5	induced	---	---	---	100%	---
	14834	29	STOP	50 nM	80%	20%
	"	"	"	200 nM	13%	87%
	21812	58	intron 1	50 nM	110%	---
	"	"	"	200 nM	193%	---
10	21833	79	intron 3	50 nM	88%	12%
	"	"	"	200 nM	8%	92%
	21834	80	intron 3	50 nM	70%	30%
	"	"	"	200 nM	18%	82%
	21835	81	intron 3	50 nM	106%	---
15	"	"	"	200 nM	42%	58%
	21836	82	intron 3	50 nM	71%	29%
	"	"	"	200 nM	12%	88%
	21837	83	intron 3	50 nM	129%	---
	"	"	"	200 nM	74%	26%
20	21838	84	intron 3	50 nM	85%	15%
	"	"	"	200 nM	41%	59%
	21839	85	intron 3	50 nM	118%	---
	"	"	"	200 nM	58%	42%
	21840	86	intron 3	50 nM	120%	---
25	"	"	"	200 nM	96%	4%
	21841	87	intron 3	50 nM	117%	---
	"	"	"	200 nM	78%	22%

TABLE 13

Nucleotide Sequences of TNF- α Chimeric (deoxy gapped) 2'-O-methoxyethyl Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE (5' -> 3')	SEQ	TARGET GENE		GENE TARGET REGION
			ID	NUCLEOTIDE NO: CO-ORDINATES ¹	
5 21725	AoGoCoGoCsTsGsASGStsCsGsGSTsCsAoCoCoT	91		2501-2520	exon 4
25655	A GGCSGSCS <small>T</small> SGsASGStsCsGsGSTsCsAsCsCScsT	"		"	"
25656	A GGCSGSCS <small>T</small> SGsASGStsCsGsGSTsCsAsCsCScsT	"		"	"
25660	AoGoCoGcsCstSgsASGStsCsGsGSTsCsAsCoCoT	"		"	"
21732	GoGotoAOGsGsASGSAsCsGsGsAstoGoCoGoG	98		2361-2380	exon 4
10 25657	GSGstsAsGSGsAsGSAsCsGsGsCsGsAstsGscsGsG	"		"	"
25658	GSGstsAsGSGsAsGSAsCsGsGsCsGsAstsGscsGsG	"		"	"
25661	GoGotoAsGsgsAsGSAsCsGsGsCsGsAstsGoCoGoG	"		"	"

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethoxy cytidines and 2'-deoxycytidines are 5-methyl-cytidines; "s" linkages are 15 phosphorothioate linkages, "o" linkages are phosphodiester linkages.

² Co-ordinates from Genbank Accession No. X02910, locus name "HSTNFA", SEQ ID NO. 1.

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TABLE 14

Dose Response of 20 Hour PMA-Induced neoHK Cells to TNF- α
 Antisense Oligonucleotides (ASOs)

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% protein Expression	% protein Inhibition
5	induced	---	---	---	100%	---
	14834	29	STOP	75 nM	91.2%	8.8%
	"	"	"	150 nM	42.0%	58.0%
	"	"	"	300 nM	16.9%	83.1%
	21820	66	intron 1	75 nM	79.0%	21.0%
10	"	"	"	150 nM	34.5%	65.5%
	"	"	"	300 nM	15.6%	84.4%
	21823	69	intron 1	75 nM	79.5%	20.5%
	"	"	"	150 nM	31.8%	68.2%
	"	"	"	300 nM	16.2%	83.8%
15	21725	91	exon 4	75 nM	74.8%	25.2%
	"	"	"	150 nM	58.4%	41.6%
	"	"	"	300 nM	45.2%	54.8%
	25655	91	exon 4	75 nM	112.0%	---
	"	"	"	150 nM	55.0%	45.0%
20	"	"	"	300 nM	39.3%	60.7%
	25656	91	exon 4	75 nM	108.3%	---
	"	"	"	150 nM	60.7%	39.3%
	"	"	"	300 nM	42.8%	57.2%
	25660	91	exon 4	75 nM	93.2%	6.8%
25	"	"	"	150 nM	72.8%	27.2%
	"	"	"	300 nM	50.3%	49.7%

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TABLE 15

Dose Response of 20 Hour PMA-Induced neoHK Cells to TNF- α
Antisense Oligonucleotides (ASOs)

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% protein Expression	% protein Inhibition
5	induced	---	---	---	100%	---
	14834	29	STOP	75 nM	44.9%	55.1%
	"	"	"	150 nM	16.3%	83.7%
	"	"	"	300 nM	2.2%	97.8%
	21834	80	intron 3	75 nM	102.9%	---
10	"	"	"	150 nM	24.5%	75.5%
	"	"	"	300 nM	19.1%	80.9%
	21836	82	intron 3	75 nM	70.8%	29.2%
	"	"	"	150 nM	55.9%	44.1%
	"	"	"	300 nM	32.7%	67.3%
15	21732	98	exon 4	75 nM	42.4%	57.6%
	"	"	"	150 nM	34.9%	65.1%
	"	"	"	300 nM	15.4%	84.6%
	25657	98	exon 4	75 nM	46.7%	53.3%
	"	"	"	150 nM	72.0%	28.0%
20	"	"	"	300 nM	50.6%	49.4%
	25658	98	exon 4	75 nM	83.7%	16.3%
	"	"	"	150 nM	56.6%	43.4%
	"	"	"	300 nM	36.9%	63.1%
	25661	98	exon 4	75 nM	54.9%	45.1%
25	"	"	"	150 nM	34.4%	65.6%
	"	"	"	300 nM	8.6%	91.4%

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EXAMPLE 7: Activity of Fully 2'-MOE Modified TNF- α Antisense Oligonucleotides

A series of antisense oligonucleotides were synthesized targeting the terminal twenty nucleotides of each exon at 5 every exon-intron junction of the TNF- α gene. These oligonucleotides were synthesized as fully 2'-methoxyethoxy modified oligonucleotides. The oligonucleotide sequences are shown in Table 16. Oligonucleotide 12345 (SEQ ID NO. 106) is an antisense oligonucleotide targeted to the human 10 intracellular adhesion molecule-1 (ICAM-1) and was used as an unrelated target control.

The oligonucleotides were screened at 50 nM and 200 nM for their ability to inhibit TNF- α mRNA levels, as described in Example 3. Results are shown in Table 17. Oligonucleotide 15 21794 (SEQ ID NO. 102) showed an effect at both doses, with greater than 75% inhibition at 200 nM.

TABLE 16
Nucleotide Sequences of Human TNF- α Uniform 2'-MOE Oligonucleotides

20	ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION ³
	21792	AGGCACTCACCTCTTCCCTC	100	0972-0991	E1/I1
	21793	CCCTGGGAACTGTTGGGA	101	1579-1598	I1/E2
	21794	AGACACTTACTGACTGCCTG	102	1625-1644	E2/I2
25	21795	GAAGATGATCCTGAAGAGGA	103	1812-1831	I2/E3
	21796	GAGCTCTTACCTACAACATG	104	1860-1879	E3/I3
	21797	TGAGGGTTGCTGGAGGGAG	105	2161-2180	I3/E4
	12345	GATCGCGTCGGACTATGAAG	106	target control	

¹ Emboldened residues are 2'-methoxyethoxy residues, 2'-methoxyethoxy cytosine residues are 5-methyl-cytosines; all 30

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linkages are phosphorothioate linkages.

² Co-ordinates from Genbank Accession No. X02910, locus name "HSTNFA", SEQ ID NO. 1.

³ Each target region is an exon-intron junction and is
5 represented in the form, for example, I1/E2, where I,
followed by a number, refers to the intron number and E,
followed by a number, refers to the exon number.

TABLE 17
Dose Response of neoHK Cells to TNF- α
10 **Antisense 2'-MOE Oligonucleotides**

ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% mRNA Expression	% mRNA Inhibition
induced	---	---	---	100%	---
12345	106	control	50 nM	121%	---
"	"	"	200 nM	134%	---
15 13393	49	control	50 nM	110%	---
"	"	"	200 nM	112%	---
14834	29	STOP	50 nM	92%	8%
"	"	"	200 nM	17%	83%
21792	100	E1/I1	50 nM	105%	---
20 "	"	"	200 nM	148%	---
21793	101	I1/E2	50 nM	106%	---
"	"	"	200 nM	172%	---
21794	102	E2/I2	50 nM	75%	25%
"	"	"	200 nM	23%	77%
25 21795	103	I2/E3	50 nM	79%	21%
"	"	"	200 nM	125%	---
21796	104	E3/I3	50 nM	56%	44%
"	"	"	200 nM	150%	---
21797	105	I3/E4	50 nM	90%	10%
30 "	"	"	200 nM	128%	---

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EXAMPLE 8: Mouse TNF- α Oligonucleotide Sequences

Antisense oligonucleotides were designed to target mouse TNF- α . Target sequence data are from the TNF- α cDNA sequence published by Semon,D. et al. (*Nucleic Acids Res.* 1987, 15, 9083-9084); Genbank accession number Y00467, provided herein as SEQ ID NO: 107. Oligonucleotides were synthesized primarily as phosphorothioate oligodeoxynucleotides. Oligonucleotide sequences are shown in Table 18. Oligonucleotide 3082 (SEQ ID NO. 141) is an antisense oligodeoxynucleotide targeted to the human intracellular adhesion molecule-1 (ICAM-1) and was used as an unrelated target control. Oligonucleotide 13108 (SEQ ID NO. 142) is an antisense oligodeoxynucleotide targeted to the herpes simplex virus type 1 and was used as an unrelated target control.

P388D1, mouse macrophage cells (obtained from American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 medium with 15% fetal bovine serum (FBS) (Life Technologies, Rockville, MD).

At assay time, cell were at approximately 90% confluency. The cells were incubated in the presence of OPTI-MEM® medium (Life Technologies, Rockville, MD), and the oligonucleotide formulated in LIPOFECTIN® (Life Technologies), a 1:1 (w/w) liposome formulation of the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-n,n,n-trimethylammonium chloride (DOTMA), and dioleoyl phosphatidylethanolamine (DOPE) in membrane filtered water. For an initial screen, the oligonucleotide concentration was 100 nM in 3 μ g/ml LIPOFECTIN®. Treatment was for four hours. After treatment, the medium was removed and the cells were further incubated in RPMI medium with 15% FBS and induced with 10 ng/ml LPS. mRNA was analyzed 2 hours post-induction with PMA.

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Total mRNA was isolated using the TOTALLY RNA™ kit (Ambion, Austin, TX), separated on a 1% agarose gel, transferred to HYBOND™-N+ membrane (Amersham, Arlington Heights, IL), a positively charged nylon membrane, and 5 probed. A TNF- α probe consisted of the 502 bp EcoRI-HindIII fragment from BBG 56 (R&D Systems, Minneapolis, MN), a plasmid containing mouse TNF- α cDNA. A glyceraldehyde 3-phosphate dehydrogenase (G3PDH) probe consisted of the 1.06 kb HindIII fragment from pHcGAP 10 (American Type Culture Collection, Manassas, VA), a plasmid containing human G3PDH cDNA. The fragments were purified from low-melting temperature agarose, as described in Maniatis, T., et al., *Molecular Cloning: A Laboratory Manual*, 1989 and labeled with REDIVUE™ 32 P-dCTP (Amersham 15 Pharmacia Biotech, Piscataway, NJ) and PRIME-A-GENE® labelling kit (Promega, Madison, WI). mRNA was quantitated by a PhosphoImager (Molecular Dynamics, Sunnyvale, CA).

Secreted TNF- α protein levels were measured using a mouse TNF- α ELISA kit (R&D Systems, Minneapolis, MN or 20 Genzyme, Cambridge, MA).

TABLE 18
Nucleotide Sequences of Mouse TNF- α Phosphorothioate Oligodeoxynucleotides

	ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
25	14846	GAGCTTCTGCTGGCTGGCTG	108	4351-4370	5' -UTR
	14847	CCTTGCTGTCCTCGCTGAGG	109	4371-4390	5' -UTR
	14848	TCATGGTGTCTTTCTGGAG	110	4511-4530	AUG
	14849	CTTTCTGTGCTCATGGTGT	111	4521-4540	AUG
30	14850	GCGGATCATGCTTCTGTGC	112	4531-4550	coding
	14851	GGGAGGCCATTGGGAACCT	113	5225-5244	junction
	14852	CGAATTTGAGAAGATGATC	114	5457-5476	junction

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14846	GAGCTTCTGCTGGCTGGCTG	108	4351-4370	5'-UTR
14853	CTCCTCCACTTGGTGGTTG	115	5799-5818	junction
14854	CCTGAGATCTTATCCAGCCT	116	6540-6559	3'-UTR
14855	CAATTACAGTCACGGCTCCC	117	6927-6946	3'-UTR
15921	CCCTTCATTCTCAAGGCACA	118	5521-5540	junction
5	15922 CACCCCTCAACCGCCCCCCC	119	5551-5570	intron
	15923 AGAGCTCTGTCTTTCTCAG	120	5581-5600	intron
	15924 CACTGCTCTGACTCTCACGT	121	5611-5630	intron
	15925 ATGAGGTCCC GG GTGGCCCC	122	5651-5670	intron
	15926 CACCCTCTGTCTTCCACAT	123	5681-5700	intron
10	15927 CTCCACATCCTGAGCCTCAG	124	5731-5750	intron
	15928 ATTGAGTCAGTGTCAACCCTC	125	5761-5780	intron
	15929 GCTGGCTCAGCCACTCCAGC	126	5821-5840	coding
	15930 TCTTGAGATCCATGCCGTT	127	5861-5880	coding
	15931 AACCCATCGGCTGGCACCAAC	128	5891-5910	coding
15	15932 GTTGAGCTCAGCCCCCTCA	129	6061-6080	coding
	15933 CTCCTCCCAGGTATATGGGC	130	6091-6110	coding
	15934 TGAGTTGGTCCCCCTTCTCC	131	6121-6140	coding
	15935 CAAAGTAGACCTGCCCGGAC	132	6181-6200	coding
	15936 ACACCCATTCCCTTCACAGA	133	6211-6230	STOP
20	15937 CATAATCCCCTTCTAACGTT	134	6321-6340	3'-UTR
	15938 CACAGAGTTGGACTCTGAGC	135	6341-6360	3'-UTR
	15939 CAGCATCTTGTGTTCTGAG	136	6381-6400	3'-UTR
	15940 CACAGTCCAGGTCACTGTCC	137	6401-6420	3'-UTR
	15941 TGATGGTGGTGCATGAGAGG	138	6423-6442	3'-UTR
25	15942 GTGAATT CGGAAAGCCCATT	139	6451-6470	3'-UTR
	15943 CCTGACC ACTCTCCCTTGCG	140	6501-6520	3'-UTR
	3082 <u>TGCATCCCCAGGCCACCAT</u>	141		target control
	13108 GCCGAGGTCCATGTCGTACG	142		target control
	C			

¹ All "C" residues are 5-methyl-cytosines except underlined

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"C" residues are unmodified cytosines; all linkages are phosphorothioate linkages.

²Co-ordinates from Genbank Accession No. Y00467, locus name "MMTNFAB", SEQ ID NO. 107.

5 Results are shown in Table 19. Oligonucleotides 14853 (SEQ ID NO. 115), 14854 (SEQ ID NO. 116), 14855 (SEQ ID NO. 117), 15921 (SEQ ID NO. 118), 15923 (SEQ ID NO. 120), 15924 (SEQ ID NO. 121), 15925 (SEQ ID NO. 122), 15926 (SEQ ID NO. 123), 15929 (SEQ ID NO. 126), 15930 (SEQ ID NO. 127), 15931 (SEQ ID NO. 128), 15932 (SEQ ID NO. 129), 15934 (SEQ ID NO. 131), 15935 (SEQ ID NO. 132), 15936 (SEQ ID NO. 133), 15937 (SEQ ID NO. 134), 15939 (SEQ ID NO. 136), 15940 (SEQ ID NO. 137), 15942 (SEQ ID NO. 139), and 15943 (SEQ ID NO. 140) gave better than 50% inhibition. Oligonucleotides 15931 (SEQ ID NO. 128), 15932 (SEQ ID NO. 129), 15934 (SEQ ID NO. 131), and 15943 (SEQ ID NO. 140) gave 75% inhibition or better.

TABLE 19

Inhibition of Mouse TNF- α mRNA expression in P388D1 Cells
by Phosphorothioate Oligodeoxynucleotides

	ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
	induced	---	---	100%	0%
	3082	141	control	129%	---
25	13664	42	control	85%	15%
	14846	108	5'-UTR	84%	16%
	14847	109	5'-UTR	88%	12%
	14848	110	AUG	60%	40%
	14849	111	AUG	75%	25%
30	14850	112	coding	67%	33%
	14851	113	junction	62%	38%
	14852	114	junction	69%	31%
	14853	115	junction	49%	51%

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	14854	116	3'-UTR	31%	69%
	14855	117	3'-UTR	39%	61%
	15921	118	junction	42%	58%
	15922	119	intron	64%	36%
5	15923	120	intron	31%	69%
	15924	121	intron	29%	71%
	15925	122	intron	30%	70%
	15926	123	intron	29%	71%
	15928	125	intron	59%	41%
	15929	126	coding	38%	62%
10	15930	127	coding	43%	57%
	15931	128	coding	23%	77%
	15932	129	coding	25%	75%
	15933	130	coding	52%	48%
	15934	131	coding	21%	79%
15	15935	132	coding	39%	61%
	15936	133	STOP	35%	65%
	15937	134	3'-UTR	45%	55%
	15938	135	3'-UTR	76%	24%
	15939	136	3'-UTR	33%	67%
20	15940	137	3'-UTR	38%	62%
	15941	138	3'-UTR	54%	46%
	15942	139	3'-UTR	42%	58%
	15943	140	3'-UTR	25%	75%

25 EXAMPLE 9: Dose response of antisense phosphorothiaote
 oligodeoxynucleotide effects on mouse TNF- α mRNA levels in
 P388D1 cells

Four of the more active oligonucleotides from the initial screen were chosen for dose response assays. These 30 include oligonucleotides 15924 (SEQ ID NO. 121), 15931 (SEQ ID NO. 128), 15934 (SEQ ID NO. 131) and 15943 (SEQ ID NO.

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140). P388D1 cells were grown, treated and processed as described in Example 8. LIPOFECTIN® was added at a ratio of 3 µg/ml per 100 nM of oligonucleotide. The control included LIPOFECTIN® at a concentration of 6 µg/ml.

5 Results are shown in Table 20. Each oligonucleotide tested showed a dose response effect with maximal inhibition about 70% or greater and IC₅₀ values less than 50 nM.

TABLE 20**Dose Response of LPS-Induced P388D1 Cells to TNF-α****10 Antisense Phosphorothioate Oligodeoxynucleotides (ASOs)**

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% mRNA Expression	% mRNA Inhibition
	induced	---	---	---	100%	---
	13108	142	control	25 nM	68%	32%
	"	"	"	50 nM	71%	29%
15	"	"	"	100 nM	64%	36%
	"	"	"	200 nM	75%	25%
	15924	121	intron	25 nM	63%	37%
	"	"	"	50 nM	49%	51%
	"	"	"	100 nM	36%	64%
20	"	"	"	200 nM	31%	69%
	15931	128	coding	25 nM	42%	58%
	"	"	"	50 nM	30%	70%
	"	"	"	100 nM	17%	83%
	"	"	"	200 nM	16%	84%
25	15934	131	coding	25 nM	37%	63%
	"	"	"	50 nM	26%	74%
	"	"	"	100 nM	13%	87%
	"	"	"	200 nM	13%	87%
	15943	140	3'-UTR	25 nM	38%	62%
30	"	"	"	50 nM	38%	62%

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"	"	"	100 nM	16%	84%
"	"	"	200 nM	16%	84%

**EXAMPLE 10: Design and Testing of 2'-O-methoxyethyl (deoxy gapped) TNF- α Antisense Oligonucleotides on TNF- α Levels in
5 P388D1 Cells**

Oligonucleotides having SEQ ID NO: 128, SEQ ID NO: 131, and SEQ ID NO: 140 were synthesized as uniformly phosphorothioate oligodeoxynucleotides or mixed phosphorothioate/phosphodiester chimeric oligonucleotides 10 having variable regions of 2'-O-methoxyethyl (2'-MOE) nucleotides and deoxynucleotides. The sequences and the oligonucleotide chemistries are shown in Table 21. All 2'-MOE cytosines were 5-methyl-cytosines.

Oligonucleotides were screened as described in Example 15 8. Results are shown in Table 22. All the oligonucleotides tested, except oligonucleotide 16817 (SEQ ID NO. 140) showed 44% or greater inhibition of TNF- α mRNA expression. Oligonucleotides 16805 (SEQ ID NO: 131), 16813 (SEQ ID NO: 140), and 16814 (SEQ ID NO: 140) showed greater 20 than 70% inhibition.

TABLE 21

Nucleotide Sequences of Mouse 2'-O-methoxyethyl (deoxy gapped) TNF- α Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID		TARGET NUCLEOTIDE NO: CO-ORDINATES ²	GENE TARGET REGION
		SEQ	TARGET GENE ID		
5	15931 AsASCS-CS-AsT-ScG-G-C-S-C-T-G-G-C-S-A-S-C-C-S-A-S-C	128	5891-5910	coding	
	16797 AoAO-Co-Cs-CS-As-T-ScG-G-C-S-C-T-G-G-C-S-A-S-C-C-S-A-C	"	5891-5910	coding	
	16798 AsASCS-CS-AsT-ScG-G-C-S-C-T-G-G-C-S-A-S-C-C-S-A-C	"	5891-5910	coding	
	16799 AoAO-Co-Co-CS-As-T-ScG-G-C-S-C-T-G-G-C-S-A-C-C-O-A-C	"	5891-5910	coding	
	16800 AsASCS-CS-AsT-ScG-G-C-S-C-T-G-G-C-S-A-S-C-C-S-A-C	"	5891-5910	coding	
10	16801 AoAO-Co-Co-Co-A-O-T-O-C-O-G-S-G-C-S-T-G-G-C-S-A-S-C-C-S-A-C	"	5891-5910	coding	
	16802 AsASCS-CS-As-T-ScG-G-C-S-C-T-G-G-C-S-A-S-C-C-S-A-C	"	5891-5910	coding	
	16803 AsASCS-CS-As-T-ScG-G-C-S-C-T-G-G-C-O-C-O-A-C-C-O-A-C	"	5891-5910	coding	
	16804 AsASCS-CS-As-T-ScG-G-C-S-C-T-G-G-C-S-A-S-C-C-S-A-C	"	5891-5910	coding	
	15934 T-G-S-A-S-G-S-T-S-G-G-S-T-S-C-C-C-S-C-S-C-T-S-T-S-C-S-C	131	6121-6140	coding	
15	16805 T-O-G-O-A-G-G-T-S-T-S-G-G-S-T-S-C-C-C-S-C-S-C-S-C-T-S-C-O-C	"	6121-6140	coding	
	16806 T-S-G-S-A-S-G-S-T-S-G-G-S-T-S-C-C-C-S-C-S-C-S-C-T-S-T-S-C-S-C	"	6121-6140	coding	
	16807 T-O-G-O-A-G-G-T-S-T-S-G-G-S-T-S-C-C-C-S-C-S-C-S-C-T-S-C-O-C	"	6121-6140	coding	
	16808 T-S-G-S-A-S-G-S-T-S-G-G-S-T-S-C-C-C-S-C-S-C-S-C-T-S-T-S-C-S-C	"	6121-6140	coding	

	16809	T o G o A o G o T o G o G T C s C s C s C s T ST S C S C	"	6121-6140	coding
	16810	T s G s A s G ST S T S G S T S C S C S C S C S C S C	"	6121-6140	coding
	16811	T s G s A s G ST S T S G S T S C S C S C S C S C S C	"	6121-6140	coding
	16812	T s G s A s G ST S T S G S T S C S C S C S C S C S C	"	6121-6140	coding
5	15943	C s C s T s G s A s C s C s A s C s C s T s C s C s T s S G S C	140	6501-6520	3'-UTR
	16813	C o C o T o G s A s C s C s A s C s C s T s C s C s T s S T O T O G C	"	6501-6520	3'-UTR
	16814	C s C s T s G s A s C s C s A s C s C s T s C s C s T s S T S G S C	"	6501-6520	3'-UTR
	16815	C o C o T o G o A s C s C s A s C s C s T s C s C s T s O T O T O G C	"	6501-6520	3'-UTR
	16816	C s C s T s G s A s C s C s A s C s C s T s C s C s T s S T S G S C	"	6501-6520	3'-UTR
10	16817	C o C o T o G o A o C o C o A o C ST S T S C S C S T S T S T S G S C	"	6501-6520	3'-UTR
	16818	C s C s T s G s A s C s C s A s C s C s T s C s C s T s S T S G S C	"	6501-6520	3'-UTR
	16819	C s C s T s G s A s C s C s A s C s C s T s C s T o C o C o C o T o T o T o G bC	"	6501-6520	3'-UTR
	16820	C s C s T s G s A s C s C s A s C s C s T s C s C s T s S T S G S C	"	6501-6520	3'-UTR

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-
 15 methoxyethoxy cytidines are 5-methyl-cytidines; "s" linkages are phosphodiester linkages,
 "O" linkages are phosphorothioate linkages.
²Coordinates from Genbank Accession No. Y00467, locus name "MMTNFAB", SEQ ID NO. 107.

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TABLE 22

Inhibition of mouse TNF- α mRNA expression in P388D1 Cells
by 2'-O-methoxyethyl (deoxy gapped) Oligonucleotides

	ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
	induced	---	---	100%	0%
5	13108	142	control	87%	13%
	15934	131	coding	28%	72%
	16797	128	coding	33%	67%
10	16798	"	coding	34%	66%
	16799	"	coding	56%	44%
	16800	"	coding	35%	65%
	16801	"	coding	34%	66%
	16802	"	coding	38%	62%
15	16803	"	coding	35%	65%
	16804	"	coding	39%	61%
	16805	131	coding	29%	71%
	16806	"	coding	31%	69%
	16807	"	coding	46%	54%
20	16808	"	coding	43%	57%
	16809	"	coding	33%	67%
	16810	"	coding	37%	63%
	16811	"	coding	40%	60%
	16812	"	coding	31%	69%
25	16813	140	3' -UTR	28%	72%
	16814	"	3' -UTR	28%	72%
	16815	"	3' -UTR	46%	54%
	16816	"	3' -UTR	49%	51%
	16817	"	3' -UTR	172%	---

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16818	"	3'-UTR	34%	66%
16819	"	3'-UTR	51%	49%
16820	"	3'-UTR	44%	56%

EXAMPLE 11: Effect of TNF- α Antisense Oligonucleotides in a

5 Murine Model for Non-Insulin-dependent Diabetes Mellitus

The db/db mouse model, a standard model for non-insulin-dependent diabetes mellitus (NIDDM; Hotamisligil, G.S., et al., *Science*, 1993, 259, 87-90), was used to assess the activity of TNF- α antisense

10 oligonucleotides on blood glucose levels and TNF- α mRNA levels in whole mice. These mice have elevated blood glucose levels and TNF- α mRNA levels compared to wild type mice. Female db/db mice and wild-type littermates were purchased from Jackson Laboratories (Bar Harbor, ME). The 15 effect on oligonucleotide 15931 (SEQ ID NO. 128) on blood glucose levels was determined. For determination of TNF- α mRNA levels, oligonucleotide 15931 (SEQ ID NO. 128), a uniformly modified phosphorothioate oligodeoxynucleotide, was compared to oligonucleotide 25302 (SEQ ID NO. 128), a 20 mixed phosphorothioate/phosphodiester chimeric oligonucleotide having regions of 2'-O-methoxyethyl (2'-MOE) nucleotides and deoxynucleotides. The sequences and chemistries are shown in Table 23. Oligonucleotide 18154 (SEQ ID NO. 143) is an antisense mixed 25 phosphorothioate/phosphodiester chimeric oligonucleotide, having regions of 2'-O-methoxyethyl (2'-MOE) nucleotides and deoxynucleotides, targeted to the human vascular cell adhesion molecule-1 (VCAM-1) and was used as an unrelated target control.

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TABLE 23

Nucleotide Sequence of TNF- α Antisense Oligonucleotide

ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
5 15931	AACCCATCGGCTGGCACCA	128	5891-5910	coding
25302	AACCCATCGGCTGGCACCA	128	5891-5910	coding
18154	TCAAGCAGTGCCACCGATCC	143		target control

¹ All 2'-methoxyethyl cytosines and 2'-deoxy cytosines residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

² Co-ordinates from Genbank Accession No. Y00467, locus name "MMTNFAB", SEQ ID NO. 107.

db/db mice, six to ten weeks old, were dosed intraperitoneally with oligonucleotide every other day for 15 2 weeks at 10 mg/kg. The mice were fasted for seven hours prior to administration of the oligonucleotide. The mice were bled via retro orbital sinus every other day, and glucose measurements were performed on the blood. Results are shown in Table 24. Oligonucleotide 15931 (SEQ ID NO. 20 128) was able to reduce blood glucose levels in db/db mice to levels comparable with wild type mice. Food intake between wild type mice, treated and untreated, did not differ. Food intake between db/db mice, treated and untreated, although higher than wild type mice, did not 25 differ significantly.

Samples of the fat (adipose) tissue from the inguinal fat pads were taken for RNA extraction. RNA was extracted according to *Current Protocols in Molecular Biology, 1997*, Ausubel, F., et al. ed., John Wiley & Sons. RNA was 30 purified using the RNA clean up procedure of the RNEASY[®] Mini kit (Qiagen, Valencia, CA). TNF- α mRNA levels were

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measured using the RIBOQUANT® kit (PharMingen, San Diego, CA) with 15 µg of RNA per lane. The probe used was from the mCK-3b Multi-Probe Template set (PharMingen, San Diego, CA) labeled with [α^{32} P]UTP (Amersham Pharmacia Biotech, 5 Piscataway, NJ). Results are shown in Table 25. Both oligonucleotide 15931 (SEQ ID NO. 128) and 25302 (SEQ ID NO. 128) were able to reduce TNF- α levels in fat, with 25302 (SEQ ID NO. 128) reducing TNF- α to nearly wild-type levels.

10

TABLE 24

Level of Blood Glucose in Normal and db/db Mice After Treatment with TNF- α Antisense Oligonucleotides

	Mouse Strain	ISIS #	SEQ ID NO:	ASO Gene Target	Time (days)	blood glucose (mg/dL)
15	wild type	---	---	---	1	140
	"	15931	128	coding	"	138
	db/db	---	---	---	1	260
	"	15931	128	coding	"	254
20	wild type	---	---	---	9	175
	"	15931	128	coding	"	163
	db/db	---	---	---	9	252
	"	15931	128	coding	"	128

TABLE 25

Level of TNF- α mRNA in Fat of db/db Mice After Treatment with TNF- α Antisense Oligonucleotides

25

ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION
wt saline	---	---	100%
db/db saline	---	---	362%

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18154	142	control	130%
15931	128	coding	210%
25302	128	coding	417%

**EXAMPLE 12: Effect of TNF- α Antisense Oligonucleotides in a
5 Murine Model for Rheumatoid Arthritis**

Collagen-induced arthritis (CIA) was used as a murine model for arthritis (Mussener,A., et al., *Clin. Exp. Immunol.*, 1997, 107, 485-493). Female DBA/1LacJ mice (Jackson Laboratories, Bar Harbor, ME) between the ages of 10 6 and 8 weeks were used to assess the activity of TNF- α antisense oligonucleotides.

On day 0, the mice were immunized at the base of the tail with 100 μ g of bovine type II collagen which is emulsified in Complete Freund's Adjuvant (CFA). On day 7, 15 a second booster dose of collagen was administered by the same route. On day 14, the mice were injected subcutaneously with 100 μ g of LPS. Oligonucleotide was administered intraperitoneally daily (10 mg/kg bolus) starting on day -3 (three days before day 0) and 20 continuing for the duration of the study.

Weights were recorded weekly. Mice were inspected daily for the onset of CIA. Paw widths are rear ankle widths of affected and unaffected joints were measured three times a week using a constant tension caliper. Limbs 25 were clinically evaluated and graded on a scale from 0-4 (with 4 being the highest).

Oligonucleotide 25302 (SEQ ID NO. 128) was compared to a saline control. The antisense TNF- α oligonucleotide reduced the incidence of CIA from 70% for the saline 30 control to 40% for the oligonucleotide. The severity of the disease (based on the mean score of the limbs) was also reduced from 3.2 for the saline control to 2.1 for the

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oligonucleotide.

EXAMPLE 13: Effect of TNF- α Antisense Oligonucleotides in a Murine Model for Contact Sensitivity

Contact sensitivity is a type of immune response resulting from contact of the surface of the skin with a sensitizing chemical. A murine model for contact sensitivity is widely used to develop therapies for chronic inflammation, autoimmune disorder, and organ transplant rejection (Goebeler,M., et al., *Int Arch. Allergy Appl. Immunol.*, 1990, 93, 294-299). One example of such a disease is atopic dermatitis. Female Balb/c mice between the ages of 8 and 12 weeks are used to assess the activity of TNF- α antisense oligonucleotides in a contact sensitivity model.

Balb/c mice receive injections of oligonucleotide drug in saline via i.v. injection into the tail vein. The abdomen of the mice is shaved using an Oster hair clipper. The animals are anesthetized using isoflurane, and 25 μ l of 0.2% 2,4-dinitrofluorobenzene (DNFB) in 4:1 acetone:olive oil is applied to the shaved abdomen two days in a row. After five days, 10 ml of 0.2% DNFB in the same vehicle is applied to the right ear. After each exposure, the mouse is suspended in air for two minutes to allow the DNFB to absorb into the skin. 24 and 48 hours after application of DNFB to the ear, the ear thickness is measured using a micrometer. Inflammation (dermatitis) is indicated by a ranked thickening of the ear. Thickness of the treated ear is compared to untreated (contralateral) ear thickness.

EXAMPLE 14: Effect of TNF- α Antisense Oligonucleotides in a Murine Model for Crohn's Disease

C3H/HeJ, SJL/JK and IL10-/- mice are used in a TNBS (2,4,5,-trinitrobenzene sulfonic acid) induced colitis model for Crohn's disease (Neurath,M.F., et al., *J. Exp. Med.*, 1995, 182, 1281-1290). Mice between the ages of 6

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weeks and 3 months are used to assess the activity of TNF- α antisense oligonucleotides.

C3H/HeJ, SJL/JK and IL10-/- mice are fasted overnight prior to administration of TNBS. A thin, flexible

5 polyethylene tube is slowly inserted into the colon of the mice so that the tip rests approximately 4 cm proximal to the anus. 0.5 mg of the TNBS in 50% ethanol is slowly injected from the catheter fitted onto a 1 ml syringe.

Animals are held inverted in a vertical position for

10 approximately 30 seconds. TNF- α antisense oligonucleotides are administered either at the first sign of symptoms or simultaneously with induction of disease.

Animals, in most cases, are dosed every day.

Administration is by i.v., i.p., s.q., minipumps or
15 intracolonic injection. Experimental tissues are collected at the end of the treatment regimen for histochemical evaluation.

EXAMPLE 15: Effect of TNF- α Antisense Oligonucleotides in a Murine Model for Multiple Sclerosis

20 Experimental autoimmune encephalomyelitis (EAE) is a commonly accepted murine model for multiple sclerosis (Myers, K.J., et al., *J. Neuroimmunol.*, 1992, 41, 1-8). SJL/H, PL/J, (SJLxPL/J)F1, (SJLxBalb/c)F1 and Balb/c female mice between the ages of 6 and 12 weeks are used to test
25 the activity of TNF- α antisense oligonucleotides.

The mice are immunized in the two rear foot pads and base of the tail with an emulsion consisting of encephalitogenic protein or peptide (according to Myers, K.J., et al., *J. of Immunol.*, 1993, 151, 2252-2260)

30 in Complete Freund's Adjuvant supplemented with heat killed Mycobacterium tuberculosis. Two days later, the mice

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receive an intravenous injection of 500 ng Bordatella pertussis toxin and additional adjuvant.

Alternatively, the disease may also be induced by the adoptive transfer of T-cells. T-cells are obtained from 5 the draining of the lymph nodes of mice immunized with encephalitogenic protein or peptide in CFA. The T cells are grown in tissue culture for several days and then injected intravenously into naive syngeneic recipients.

Mice are monitored and scored daily on a 0-5 scale for 10 signals of the disease, including loss of tail muscle tone, wobbly gait, and various degrees of paralysis.

EXAMPLE 16: Effect of TNF- α Antisense Oligonucleotides in a Murine Model for Pancreatitis

Swiss Webster, C57BL/56, C57BL/6 lpr and gld male mice 15 are used in an experimental pancreatitis model (Niederau,C., et al., *Gastroenterology*, 1985, 88, 1192-1204). Mice between the ages of 4 and 10 weeks are used to assess the activity of TNF- α antisense oligonucleotides.

Caerulein (5-200 μ g/kg) is administered i.p. every hour 20 for one to six hours. At varying time intervals, the mice are given i.p. injection of avertin and bled by cardiac puncture. The pancreas and spleen are evaluated for histopathology and increased levels of IL-1 β , IL-6, and TNF- α . The blood is analyzed for increased levels of serum 25 amylase and lipase. TNF- α antisense oligonucleotides are administered by intraperitoneal injection at 4 hours pre-caerulein injections.

EXAMPLE 17: Effect of TNF- α Antisense Oligonucleotides in a Murine Model for Hepatitis

Concanavalin A-induced hepatitis is used as a murine 30 model for hepatitis (Mizuhara,H., et al., *J. Exp. Med.*, 1994, 179, 1529-1537). It has been shown that this type of liver injury is mediated by Fas (Seino,K., et al.,

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(*Gastroenterology* 1997, 113, 1315-1322). Certain types of viral hepatitis, including Hepatitis C, are also mediated by Fas (*J. Gastroenterology and Hepatology*, 1997, 12, S223-S226). Female Balb/c and C57BL/6 mice between the ages of 5 6 weeks and 3 months are used to assess the activity of TNF- α antisense oligonucleotides.

Mice are intravenenously injected with oligonucleotide. The pretreated mice are then intravenously injected with 0.3 mg concanavalin A (Con A) 10 to induce liver injury. Within 24 hours following Con A injection, the livers are removed from the animals and analyzed for cell death (apoptosis) by *in vitro* methods. In some experiments, blood is collected from the retro-orbital vein.

15 **EXAMPLE 18: Effect of Antisense Oligonucleotide Targeted to TNF- α on Survival in Murine Heterotopic Heart Transplant Model**

To determine the therapeutic effects of TNF- α antisense oligonucleotides in preventing allograft 20 rejection, murine TNF- α -specific oligonucleotides are tested for activity in a murine vascularized heterotopic heart transplant model. Hearts from Balb/c mice are transplanted into the abdominal cavity of C3H mice as primary vascularized grafts essentially as described by Isobe 25 et al., *Circulation* 1991, 84, 1246-1255. Oligonucleotide is administered by continuous intravenous administration via a 7-day Alzet pump. The mean survival time for untreated mice is usually approximately 9-10 days.

Treatment of the mice for 7 days with TNF- α antisense 30 oligonucleotides is expected to increase the mean survival time.

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EXAMPLE 19: Optimization of Human TNF- α Antisense Oligonucleotide

Additional antisense oligonucleotides targeted to intron 1 of human TNF- α were designed. These are shown in 5 Table 26. Oligonucleotides are screened by RT-PCR as described in Example 5 hereinabove.

TABLE 26

Nucleotide Sequences of Human TNF- α Intron 1 Antisense Oligonucleotides

10	ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
	100181	AGTGTCTTCTGTGTGCCAGA	144	1409-1428	intron 1
	100201	AGTGTCTTCTGTGTGCCAGA	"	"	intron 1
	100230	AGTGTCTTCTGTGTGCCAGA	"	"	intron 1
15	100250	AGTGTCTTCTGTGTGCCAGA	"	"	intron 1
	100182	GTGTCTTCTGTGTGCCAGAC	145	1408-1427	intron 1
	100202	GTGTCTTCTGTGTGCCAGAC	"	"	intron 1
	100231	GTGTCTTCTGTGTGCCAGAC	"	"	intron 1
	100251	GTGTCTTCTGTGTGCCAGAC	"	"	intron 1
20	100183	TGTCTTCTGTGTGCCAGACA	146	1407-1426	intron 1
	100203	TGTCTTCTGTGTGCCAGACA	"	"	intron 1
	100232	TGTCTTCTGTGTGCCAGACA	"	"	intron 1
	100252	TGTCTTCTGTGTGCCAGACA	"	"	intron 1
	100184	GTCTTCTGTGTGCCAGACAC	147	1406-1425	intron 1
25	100204	GTCTTCTGTGTGCCAGACAC	"	"	intron 1
	100233	GTCTTCTGTGTGCCAGACAC	"	"	intron 1
	100253	GTCTTCTGTGTGCCAGACAC	"	"	intron 1
	100185	TCTTCTGTGTGCCAGACACC	148	1405-1424	intron 1
	100205	TCTTCTGTGTGCCAGACACC	"	"	intron 1
30	100234	TCTTCTGTGTGCCAGACACC	"	"	intron 1

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	100254	TCTTCTGTGTGCCAGACACCC	"	"	intron 1
	100186	CTTCTGTGTGCCAGACACCC	149	1404-1423	intron 1
	100206	CTTCTGTGTGCCAGACACCC	"	"	intron 1
	100235	CTTCTGTGTGCCAGACACCC	"	"	intron 1
5	100255	CTTCTGTGTGCCAGACACCC	"	"	intron 1
	100187	TTCTGTGTGCCAGACACCC	150	1403-1422	intron 1
	100207	TTCTGTGTGCCAGACACCC	"	"	intron 1
	100236	TTCTGTGTGCCAGACACCC	"	"	intron 1
	100256	TTCTGTGTGCCAGACACCC	"	"	intron 1
10	100188	TCTGTGTGCCAGACACCC	151	1402-1421	intron 1
	100208	TCTGTGTGCCAGACACCC	"	"	intron 1
	100237	TCTGTGTGCCAGACACCC	"	"	intron 1
	100257	TCTGTGTGCCAGACACCC	"	"	intron 1
	100189	CTGTGTGCCAGACACCC	152	1401-1420	intron 1
15	100209	CTGTGTGCCAGACACCC	"	"	intron 1
	100238	CTGTGTGCCAGACACCC	"	"	intron 1
	100258	CTGTGTGCCAGACACCC	"	"	intron 1
	100190	TGTGTGCCAGACACCC	153	1400-1419	intron 1
	100210	TGTGTGCCAGACACCC	"	"	intron 1
20	100239	TGTGTGCCAGACACCC	"	"	intron 1
	100259	TGTGTGCCAGACACCC	"	"	intron 1
	100191	TGTGCCAGACACCTATCTT	154	1398-1417	intron 1
	100211	TGTGCCAGACACCTATCTT	"	"	intron 1
	100240	TGTGCCAGACACCTATCTT	"	"	intron 1
25	100260	TGTGCCAGACACCTATCTT	"	"	intron 1
	100192	GTGCCAGACACCTATCTC	155	1397-1416	intron 1
	100212	GTGCCAGACACCTATCTC	"	"	intron 1
	100241	GTGCCAGACACCTATCTC	"	"	intron 1
	100261	GTGCCAGACACCTATCTC	"	"	intron 1

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	100193	TGCCAGACACCCCTATCTTCT	156	1396-1415	intron 1
	100213	TGCCAGACACCCCTATCTTCT	"	"	intron 1
	100242	TGCCAGACACCCCTATCTTCT	"	"	intron 1
	100262	TGCCAGACACCCCTATCTTCT	"	"	intron 1
5	100194	GCCAGACACCCCTATCTTCTT	157	1395-1414	intron 1
	100214	GCCAGACACCCCTATCTTCTT	"	"	intron 1
	100243	GCCAGACACCCCTATCTTCTT	"	"	intron 1
	100263	GCCAGACACCCCTATCTTCTT	"	"	intron 1
	100195	CCAGACACCCCTATCTTCTTC	158	1394-1413	intron 1
10	100215	CCAGACACCCCTATCTTCTTC	"	"	intron 1
	100244	CCAGACACCCCTATCTTCTTC	"	"	intron 1
	100264	CCAGACACCCCTATCTTCTTC	"	"	intron 1
	100196	CAGACACCCCTATCTTCTTCT	159	1393-1412	intron 1
	100216	CAGACACCCCTATCTTCTTCT	"	"	intron 1
15	100245	CAGACACCCCTATCTTCTTCT	"	"	intron 1
	100265	CAGACACCCCTATCTTCTTCT	"	"	intron 1
	100197	AGACACCCCTATCTTCTTCTC	160	1392-1411	intron 1
	100217	AGACACCCCTATCTTCTTCTC	"	"	intron 1
	100246	AGACACCCCTATCTTCTTCTC	"	"	intron 1
20	100266	AGACACCCCTATCTTCTTCTC	"	"	intron 1
	100198	GACACCCCTATCTTCTTCTCT	161	1391-1410	intron 1
	100218	GACACCCCTATCTTCTTCTCT	"	"	intron 1
	100247	GACACCCCTATCTTCTTCTCT	"	"	intron 1
	100267	GACACCCCTATCTTCTTCTCT	"	"	intron 1
25	100199	ACACCCCTATCTTCTTCTCTC	162	1390-1409	intron 1
	100219	ACACCCCTATCTTCTTCTCTC	"	"	intron 1
	100248	ACACCCCTATCTTCTTCTCTC	"	"	intron 1
	100268	ACACCCCTATCTTCTTCTCTC	"	"	intron 1
	100200	CACCCCTATCTTCTTCTCTCC	163	1389-1408	intron 1

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100220	CACCCATATCTTCTTCTCTCC	"	"	intron 1
100249	CACCCATATCTTCTTCTCTCC	"	"	intron 1
100269	CACCCATATCTTCTTCTCTCC	"	"	intron 1
100270	GTCTTCTGTGTGCCAGAC	164	1408-1425	intron 1
5	TCTTCTGTGTGCCAGACA	165	1407-1424	intron 1
100272	CTTCTGTGTGCCAGACAC	166	1406-1423	intron 1
100273	TTCTGTGTGCCAGACACC	167	1405-1422	intron 1
100274	TCTGTGTGCCAGACACCC	168	1404-1421	intron 1
100275	CTGTGTGCCAGACACCC	169	1403-1420	intron 1
10	TGTGTGCCAGACACCC	170	1402-1419	intron 1
100277	GTGTGCCAGACACCC	171	1401-1418	intron 1
100278	TGTGCCAGACACCC	172	1400-1417	intron 1
100279	TGCCAGACACCC	173	1398-1415	intron 1
100280	GCCAGACACCC	174	1397-1414	intron 1
15	CCAGACACCC	175	1396-1413	intron 1
100282	CAGACACCC	176	1395-1412	intron 1
100283	AGACACCC	177	1394-1411	intron 1
100284	GACACCC	178	1393-1410	intron 1
100285	ACACCC	179	1392-1409	intron 1

20 ¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethyl cytosines and 2'-deoxy cytosines residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

25 ²Co-ordinates from Genbank Accession No. X02910, locus name "HSTNFA", SEQ ID NO. 1.

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EXAMPLE 20: Design of Antisense Oligonucleotides Targeting Human TNF- α Intron 2

Additional antisense oligonucleotides targeted to intron 2 and coding regions of human TNF- α were designed.

- 5 These are shown in Table 27. Oligonucleotides are screened by RT-PCR as described in Example 5 hereinabove.

TABLE 27

Nucleotide Sequences of Human TNF- α Intron 2 Antisense Oligonucleotides

	ISIS No.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
	100549	AGAGGTTGGAGACACTTAC	180	1635-1654	intron 2
	100566	AGAGGTTGGAGACACTTAC	"	"	intron 2
	100550	GAATTAGGAAAGAGGTTGG	181	1645-1664	intron 2
15	100567	GAATTAGGAAAGAGGTTGG	"	"	intron 2
	100551	CCCAAACCCAGAATTAGGAA	182	1655-1674	intron 2
	100568	CCCAAACCCAGAATTAGGAA	"	"	intron 2
	100552	TACCCCCAACCCAAACCCA	183	1665-1684	intron 2
	100569	TACCCCCAACCCAAACCCA	"	"	intron 2
20	100553	GTACTAACCTACCCCAA	184	1675-1694	intron 2
	100570	GTACTAACCTACCCCAA	"	"	intron 2
	100554	TTCCATACCGGTACTAACCC	185	1685-1704	intron 2
	100571	TTCCATACCGGTACTAACCC	"	"	intron 2
	100555	CCCCCACTGCTTCATACCG	186	1695-1714	intron 2
25	100572	CCCCCACTGCTTCATACCG	"	"	intron 2
	100556	CTTTAAATTCCCCACTGC	187	1705-1724	intron 2
	100573	CTTTAAATTCCCCACTGC	"	"	intron 2
	100557	AAGACCAAAACTTAAATT	188	1715-1734	intron 2
	100571	AAGACCAAAACTTAAATT	"	"	intron 2
30	100558	ATCCTCCCCAAGACAAAA	189	1725-1744	intron 2

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100640	ATCCTCCCCAAGACCAAA	"	"	intron 2
100559	ACCTCCATCCATCCTCCCC	190	1735-1754	intron 2
100641	ACCTCCATCCATCCTCCCC	"	"	intron 2
100560	CCCTACTTACCTCCATCC	191	1745-1764	intron 2
5	100642 CCCTACTTACCTCCATCC	"	"	intron 2
100561	GAAAATACCCCCCTACTTTC	192	1755-1774	intron 2
100643	GAAAATACCCCCCTACTTTC	"	"	intron 2
100562	AAACTTCCTAGAAAATACCC	193	1765-1784	intron 2
100644	AAACTTCCTAGAAAATACCC	"	"	intron 2
10	100563 TGAGACCCTTAAACTTCCTA	194	1775-1794	intron 2
100645	TGAGACCCTTAAACTTCCTA	"	"	intron 2
100564	AAGAAAAAGCTGAGACCCTT	195	1785-1804	intron 2
100646	AAGAAAAAGCTGAGACCCTT	"	"	intron 2
100565	GGAGAGAGAAAAGAAAAAGC	196	1795-1814	intron 2
15	100647 GGAGAGAGAAAAGAAAAAGC	"	"	intron 2
100575	TGAGCCAGAACAGAGGTTGAGG	197	2665-2684	coding
100576	ATTCTCTTTTGAGCCAGAA	198	2675-2694	coding
100577	TAAGCCCCAATTCTCTTT	199	2685-2704	coding
100578	GTTCCGACCCCTAACGCCCA	200	2695-2714	coding
20	100579 CTAAGCTGGGTTCCGACCC	201	2705-2724	coding
100580	GCTTAAAGTTCTAACGCTTGG	202	2715-2734	coding
100581	TGGTCTTGGTCTTAAAGTT	203	2725-2744	coding
100582	TTCGAAGTGGTGGTCTTGTT	204	2735-2754	coding
100583	AATCCCAGGTTTCGAAGTGG	205	2745-2764	coding
25	100584 CACATTCTGAATCCCAGGT	206	2755-2774	coding
100585	GTGCAGGCCACACATTCTG	207	2765-2784	coding
100586	GCACCTCACTGTGCAGGCCA	208	2775-2794	coding
100587	GTGGTTGCCAGCACCTCACT	209	2785-2804	coding
100588	TGAATTCTTAGTGGTTGCCA	210	2795-2814	coding
30	100589 GGCCCCAGTTGAATTCTTA	211	2805-2824	coding

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100590	GAGTTCTGGAGGCCAGTT	212	2815-2834	coding
100591	AGGCCCGAGTGAGTTCTGGA	32	2825-2844	coding
100592	TCAAAGCTGTAGGCCAGT	214	2835-2854	coding
100593	ATGTCAGGGATCAAAGCTGT	215	2845-2864	coding
5	CAGATTCCAGATGTCAGGGA	216	2855-2874	coding
100595	CCCTGGTCTCCAGATTCCAG	217	2865-2884	coding
100596	ACCAAAGGCTCCCTGGTCTC	218	2875-2894	coding
100597	TCTGGCCAGAACCAAAGGCT	219	2885-2904	coding
100598	CCTGCAGCATTCTGCCAGA	220	2895-2914	coding
10	CTTCTCAAGTCCTGCAGCAT	221	2905-2924	coding
100600	TAGGTGAGGTCTTCTCAAGT	222	2915-2934	coding
100601	TGTCAATTCTAGGTGAGGT	223	2925-2944	coding
100602	GGTCCACTTGTGTCAATTTC	224	2935-2954	coding
100603	GAAGGCCTAACGGTCCACTTG	225	2945-2964	coding
15	CTGGAGAGAGGAAGGCCTAA	226	2955-2974	coding
100605	CTGGAAACATCTGGAGAGAG	227	2965-2984	coding
100606	TCAAGGAAGTCTGGAAACAT	228	2975-2994	coding
100607	GCTCCGTCTCAAGGAAGT	229	2985-3004	coding
100608	ATAAAATACATTCATCTGTAA	230	3085-3104	coding
20	GGTCTCCCAAATAAATACAT	231	3095-3114	coding
100610	AGGATACCCCGGTCTCCCAA	232	3105-3124	coding
100611	TGGGTCCCCCAGGATACCCC	35	3115-3134	coding
100612	GCTCCTACATTGGGTCCCCC	234	3125-3144	coding
100613	AGCCAAGGCAGCTCCTACAT	235	3135-3154	coding
25	AACATGTCTGAGCCAAGGCA	236	3145-3164	coding
100615	TTTCACGGAAAACATGTCTG	237	3155-3174	coding
100616	TCAGCTCCGTTTCACGGAA	238	3165-3184	coding
100617	AGCCTATTGTTCAGCTCCGT	239	3175-3194	coding
100618	ACATGGAACAGCCTATTGT	240	3185-3204	coding

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100619	ATCAAAAGAAGGCACAGAGG	241	3215-3234	coding
100620	GTTTAGACAACCTAACAGA	242	3255-3274	coding
100621	AATCAGCATTGTTAGACAA	243	3265-3284	coding
100622	TTGGTCACCAAATCAGCATT	244	3275-3294	coding
5	TGAGTGACAGTTGGTCACCA	245	3285-3304	coding
100624	GGCTCAGCAATGAGTGACAG	246	3295-3314	coding
100625	ATTACAGACACAACTCCCCT	247	3325-3344	coding
100626	TAGTAGGGCGATTACAGACA	248	3335-3354	coding
100627	CGCCACTGAATAGTAGGGCG	249	3345-3364	coding
10	CTTTATTTCTGCCACTGAA	250	3355-3374	coding

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethyl cytosines and 2'-deoxy cytosines residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

15 ² Co-ordinates from Genbank Accession No. X02910, locus name "HSTNFA", SEQ ID NO. 1.

Several of these oligonucleotides were chosen for dose response studies. Cells were grown and treated as described in Example 3. Results are shown in Table 28.

20 Each oligonucleotide tested showed a dose response curve with maximum inhibition greater than 75%.

TABLE 28

**Dose Response of PMA-Induced neoHK Cells to TNF- α
Antisense Oligonucleotides (ASOs)**

25	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% protein Expression	% protein Inhibition
	induced	---	---	---	100%	---
	100235	149	intron 1	75 nM	77%	23%
	"	"	"	150 nM	25%	75%

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	"	"	"	300 nM	6%	94%
100243	157	intron 1	75 nM	68%	32%	
"	"	"	150 nM	15%	85%	
"	"	"	300 nM	6%	94%	
5	100263	157	intron 1	75 nM	79%	21%
"	"	"	150 nM	30%	70%	
"	"	"	300 nM	23%	77%	

EXAMPLE 21: Optimization of Human TNF- α Antisense Oligonucleotide Chemistry

10 Analogs of oligonucleotides 21820 (SEQ ID NO. 66) and 21823 (SEQ ID NO. 69) were designed and synthesized to find an optimum gap size. The sequences and chemistries are shown in Table 29.

15 Dose response experiments were performed as described in Example 3. Results are shown in Table 30.

TABLE 29

Nucleotide Sequences of TNF- α Chimeric Backbone (deoxy gapped) Oligonucleotides

	ISIS NO.	NUCLEOTIDE SEQUENCE (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE CO- ORDINATES ¹	GENE TARGET REGION
20	21820	ATATTTCCCGCTCTTCTGT	66	1339-1358	intron 1
	28086	ATATTTCCCGCTCTTCTGT	"	"	"
	28087	ATATTTCCCGCTCTTCTGT	"	"	"
	21823	GTGTGCCAGACACCCTATCT	69	1399-1418	intron 1
25	28088	GTGTGCCAGACACCCTATCT	"	"	"
	28089	GTGTGCCAGACACCCTATCT	"	"	"

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethoxy cytidines and 2'-

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deoxycytidines are 5-methyl-cytidines; all linkages are phosphorothioate linkages.

² Co-ordinates from Genbank Accession No. X02910, locus name "HSTNFA", SEQ ID NO. 1.

5

TABLE 30

Dose Response of 20 Hour PMA-Induced neoHK Cells to TNF- α Chimeric (deoxy gapped) Antisense Oligonucleotides (ASOs)

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% protein Expression	% protein Inhibition
	induced	---	---	---	100%	---
10	13393	49	control	75 nM	150.0%	---
	"	"	"	150 nM	135.0%	---
	"	"	"	300 nM	90.0%	10.0%
15	21820	66	intron 1	75 nM	65.0%	35.0%
	"	"	"	150 nM	28.0%	72.0%
	"	"	"	300 nM	9.7%	90.3%
20	28086	66	intron 1	75 nM	110.0%	---
	"	"	"	150 nM	83.0%	17.0%
	"	"	"	300 nM	61.0%	39.0%
25	28087	66	intron 1	75 nM	127.0%	---
	"	"	"	150 nM	143.0%	---
	"	"	"	300 nM	147.0%	---
30	21823	69	intron 1	75 nM	35.0%	65.0%
	"	"	"	150 nM	30.0%	70.0%
	"	"	"	300 nM	6.4%	93.6%
25	28088	69	intron 1	75 nM	56.0%	44.0%
	"	"	"	150 nM	26.0%	74.0%
	"	"	"	300 nM	11.0%	89.0%
28089	28089	69	intron 1	75 nM	76.0%	24.0%
	"	"	"	150 nM	53.0%	47.0%
30	"	"	"	300 nM	23.0%	77.0%

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EXAMPLE 22: Screening of additional TNF- α chimeric (deoxy gapped) antisense oligonucleotides

Additional oligonucleotides targeting the major regions of TNF- α were synthesized. Oligonucleotides were 5 synthesized as uniformly phosphorothioate chimeric oligonucleotides having regions of five 2'-O-methoxyethyl (2'-MOE) nucleotides at the wings and a central region of ten deoxynucleotides. Oligonucleotide sequences are shown in Table 31.

10 Oligonucleotides were screened as described in Example 5. Results are shown in Table 32.

TABLE 31

Nucleotide Sequence of Additional Human TNF- α Chimeric (deoxy gapped) Antisense Oligonucleotides

	ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
15	104649	CTGAGGGAGCGTCTGCTGGC	251	0616-0635	5' -UTR
	104650	CCTTGCTGAGGGAGCGTCTG	252	0621-0640	5' -UTR
	104651	CTGGTCCTCTGCTGTCCTTG	253	0636-0655	5' -UTR
20	104652	CCTCTGCTGTCCTTGCTGAG	254	0631-0650	5' -UTR
	104653	TTCTCTCCCTCTTAGCTGGT	255	0651-0670	5' -UTR
	104654	TCCCTCTTAGCTGGTCCTCT	256	0646-0665	5' -UTR
	104655	TCTGAGGGTTGTTTCAGGG	257	0686-0705	5' -UTR
	104656	CTGTAGTTGCTTCTCTCCCT	258	0661-0680	5' -UTR
25	104657	ACCTGCCTGGCAGCTTGTCA	259	0718-0737	5' -UTR
	104658	GGATGTGGCGTCTGAGGGTT	260	0696-0715	5' -UTR
	104659	TGTGAGAGGAAGAGAACCTG	261	0733-0752	5' -UTR
	104660	GAGGAAGAGAACCTGCCTGG	262	0728-0747	5' -UTR
	104661	AGCCGTGGGTCACTATGTGA	263	0748-0767	5' -UTR
30	104662	TGGGTCAAGTATGTGAGAGGA	264	0743-0762	5' -UTR

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	104663	GAGAGGGTGAAGCCGTGGGT	265	0758-0777	5' -UTR
	104664	TCATGGTGTCTTCCAGGG	266	0780-0799	AUG
	104665	CTTTCAGTGCTCATGGTGTG	267	0790-0809	AUG
	104666	TCATGCTTCAGTGCTCATG	268	0795-0814	AUG
5	104667	ACGTCCCAGGATCATGCTTTC	269	0805-0824	coding
	104668	GCTCCACGTCCCAGGATCATG	270	0810-0829	coding
	104669	TCCTCGGCCAGCTCCACGTC	271	0820-0839	coding
	104670	GCGCCTCCTCGGCCAGCTCC	272	0825-0844	coding
	104671	AGGAACAAGCACCGCCTGGA	273	0874-0893	coding
10	104672	CAAGCACCGCCTGGAGCCCT	274	0869-0888	coding
	104673	AAGGAGAAGAGGCTGAGGAA	275	0889-0908	coding
	104674	GAAGAGGCTGAGGAACAAGC	276	0884-0903	coding
	104675	CCTGCCACGATCAGGAAGGA	277	0904-0923	coding
	104676	CACGATCAGGAAGGAGAAGA	278	0899-0918	coding
15	104677	AAGAGCGTGGTGGCGCCTGC	279	0919-0938	coding
	104678	CGTGGTGGCGCCTGCCACGA	280	0914-0933	coding
	104679	AAGTGCAGCAGGCAGAACAGAG	281	0934-0953	coding
	104680	CAGCAGGCAGAACAGAGCGTGG	282	0929-0948	coding
	104681	GATCACTCCAAAGTGCAGCA	283	0944-0963	coding
20	104682	GGGCCGATCACTCCAAAGTG	284	0949-0968	coding
	104683	GGGCCAGAGGGCTGATTAGA	285	1606-1625	coding
	104684	AGAGGGCTGATTAGAGAGAG	286	1601-1620	coding
	104685	GCTACAGGCTTGTCACTCGG	287	1839-1858	coding
	104686	CTGACTGCCTGGGCCAGAGG	288	1616-1635	E2/I2 ³
25	104687	TACAACATGGGCTACAGGCT	289	1849-1868	coding
	104688	AGCCACTGGAGCTGCCCTC	290	2185-2204	coding
	104689	CTGGAGCTGCCCTCAGCTT	291	2180-2199	coding
	104690	TTGGCCCGGCGGTTCAGCCA	292	2200-2219	coding
	104691	TTGGCCAGGAGGGCATTGGC	293	2215-2234	coding

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	104692	CCGGCGGTTCAGCCACTGGA	294	2195-2214	coding
	104693	CTCAGCTCCACGCCATTGGC	295	2230-2249	coding
	104694	CAGGAGGGCATTGGCCCCGGC	296	2210-2229	coding
	104695	CTCCACGCCATTGCCAGGA	297	2225-2244	coding
5	104696	ACCAGCTGGTTATCTCTCAG	298	2245-2264	coding
	104697	CTGGTTATCTCTCAGCTCCA	299	2240-2259	coding
	104698	CCCTCTGATGGCACCCACCAG	300	2260-2279	coding
	104699	TGATGGCACCAACCAGCTGGT	301	2255-2274	coding
	104700	TAGATGAGGTACAGGCCCTC	302	2275-2294	coding
10	104701	AAGAGGACCTGGGAGTAGAT	303	2290-2309	coding
	104702	GAGGTACAGGCCCTTGATG	304	2270-2289	coding
	104703	CAGCCTTGGCCCTTGAAGAG	305	2305-2324	coding
	104704	GACCTGGGAGTAGATGAGGT	306	2285-2304	coding
	104705	TTGGCCCTTGAAGAGGACCT	307	2300-2319	coding
15	104706	TGGTGTGGGTGAGGAGCACA	308	2337-2356	coding
	104707	CGGCGATGCGGCTGATGGTG	309	2352-2371	coding
	104708	TGGGTGAGGAGCACATGGGT	310	2332-2351	coding
	104709	TGGTCTGGTAGGAGACGGCG	311	2367-2386	coding
	104710	ATGC GGCTGATGGTGTGGGT	312	2347-2366	coding
20	104711	AGAGGAGGTTGACCTTGGTC	313	2382-2401	coding
	104712	TGGTAGGAGACGGCGATGCG	314	2362-2381	coding
	104713	AGGTTGACCTTGGTCTGGTA	315	2377-2396	coding
	104714	GGCTCTTGATGGCAGAGAGG	316	2397-2416	coding
	104715	TCATACCAGGGCTTGGCCTC	317	2446-2465	coding
25	104716	TTGATGGCAGAGAGGGAGGTT	318	2392-2411	coding
	104717	CCCAGATAGATGGGCTCATA	93	2461-2480	coding
	104718	CCAGGGCTTGGCCTCAGCCC	94	2441-2460	coding
	104719	AGCTGGAAGACCCCTCCCAG	319	2476-2495	coding
	104720	ATAGATGGGCTCATACCAGG	320	2456-2475	coding

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	104721	CGGTCACCCCTCTCCAGCTG	321	2491-2510	coding
	104722	GAAGACCCCTCCCAGATAGA	322	2471-2490	coding
	104723	ATCTCAGCGCTGAGTCGGTC	26	2506-2525	coding
	104724	ACCCTTCTCCAGCTGGAAGA	323	2486-2505	coding
5	104725	TAGTCGGGCCGATTGATCTC	90	2521-2540	coding
	104726	AGCGCTGAGTCGGTCACCCT	91	2501-2520	coding
	104727	TCGGCAAAGTCGAGATAGTC	324	2536-2554	coding
	104728	GGGCCGATTGATCTCAGCGC	325	2516-2535	coding
	104729	TAGACCTGCCAGACTCGGC	326	2551-2570	coding
10	104730	AAAGTCGAGATAGTCGGCC	327	2531-2550	coding
	104731	GCAATGATCCAAAGTAGAC	328	2566-2585	coding
	104732	CTGCCAGACTCGGCAAAGT	329	2546-2565	coding
	104733	CGTCCTCCTCACAGGGCAAT	330	2581-2600	stop
	104734	GATCCCAAAGTAGACCTGCC	88	2561-2580	coding
15	104735	GGAAGGTTGGATGTTCGTCC	331	2596-2615	3'-UTR
	104736	TCCTCACAGGGCAATGATCC	332	2576-2595	stop
	104737	GTTGAGGGTGTCTGAAGGAG	333	2652-2671	3'-UTR
	104738	GTTGGATGTTCGTCCTCCTC	334	2591-2610	stop
	104739	TTTGAGCCAGAACAGAGGTTGA	335	2667-2686	3'-UTR
20	104740	GAGGCCGTTGGAAAGGTTGG	336	2606-2625	3'-UTR
	104741	GCCCCCAATTCTCTTTTGA	337	2682-2701	3'-UTR
	104742	GCCAGAACAGGTTGAGGGTG	338	2662-2681	3'-UTR
	104743	GGGTTCCGACCTAAGCCCC	339	2697-2716	3'-UTR
	104744	CAATTCTTTTGAGCCAG	340	2677-2696	3'-UTR
25	104745	TAAAGTTCTAACGTTGGGTT	341	2712-2731	3'-UTR
	104746	CCGACCCCTAACGCCCCAATT	342	2692-2711	3'-UTR
	104747	GGTGGTCTTGGCTTAAAG	343	2727-2746	3'-UTR
	104748	TTCTAACGCTTGGGTTCCGAC	344	2707-2726	3'-UTR
	104749	CCCAGGTTCGAACGTGGTGG	345	2742-2761	3'-UTR

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104750	TCTTGTGCTTAAAGTTCTA	346	2722-2741	3'-UTR
104751	CACACATTCCCTGAATCCCAG	347	2757-2776	3'-UTR
104752	GTTTCGAAGTGGTGGTCTTG	348	2737-2756	3'-UTR
104753	CTTCACTGTGCAGGCCACAC	349	2772-2791	3'-UTR
5	ATTCCCTGAATCCCAGGTTTC	350	2752-2771	3'-UTR
104755	TAGTGGTTGCCAGCACATTCA	351	2787-2806	3'-UTR
104756	CCCAGTTGAATTCTTAGTG	352	2802-2821	3'-UTR
104757	CTGTGCAGGCCACACATTCC	353	2767-2786	3'-UTR
104758	GTGAGTTCTGGAGGCCAG	354	2817-2836	3'-UTR
10	GTTGCCAGCCTTCACTGTG	355	2782-2801	3'-UTR
104760	TTTGAATTCTTAGTGGTTGC	356	2797-2816	3'-UTR
104761	AAGCTGTAGGCCAGTGAG	357	2832-2851	3'-UTR
104762	TTCTGGAGGCCAGTTGA	358	2812-2831	3'-UTR
104763	AGATGTCAGGGATCAAAGCT	359	2847-2866	3'-UTR
15	TGGTCTCCAGATTCCAGATG	360	2862-2881	3'-UTR
104765	GTAGGCCAGTGAGTTCTG	361	2827-2846	3'-UTR
104766	GAACCAAAGGCTCCCTGGTC	362	2877-2896	3'-UTR
104767	TCAGGGATCAAAGCTGTAGG	363	2842-2861	3'-UTR
104768	TCCAGATTCCAGATGTCAGG	364	2857-2876	3'-UTR
20	GCAGCATTCTGGCCAGAAC	365	2892-2911	3'-UTR
104770	GTCTTCTCAAGTCCTGCAGC	366	2907-2926	3'-UTR
104771	AAAGGCTCCCTGGTCTCCAG	367	2872-2891	3'-UTR
104772	CAATTCTAGGTGAGGTCTT	368	2922-2941	3'-UTR
104773	ATTCTGGCCAGAACCAAAGG	369	2887-2906	3'-UTR
25	CTCAAGTCCTGCAGCATTCT	370	2902-2921	3'-UTR
104775	AAGGTCCACTTGTGTCAATT	370	2937-2956	3'-UTR
104776	GAGAGAGGAAGGCCTAAGGT	371	2952-2971	3'-UTR
104777	TCTAGGTGAGGTCTTCTCAA	372	2917-2936	3'-UTR
104778	CCACTTGTGTCAATTCTAG	373	2932-2951	3'-UTR

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	104779	GTCTGGAAACATCTGGAGAG	374	2967-2986	3'-UTR
	104780	CCGTGTCTCAAGGAAGTCTG	375	2982-3001	3'-UTR
	104781	AGGAAGGCCTAAGGTCCACT	376	2947-2966	3'-UTR
	104782	GAGGGAGCTGGCTCCATGGG	377	3014-3033	3'-UTR
5	104783	GAAACATCTGGAGAGAGGAA	378	2962-2981	3'-UTR
	104784	GTGCAAACATAAATAGAGGG	379	3029-3048	3'-UTR
	104785	TCTCAAGGAAGTCTGGAAAC	380	2977-2996	3'-UTR
	104786	AATAAATAATCACAAGTGCA	381	3044-3063	3'-UTR
	104787	GGGCTGGGCTCCGTGTCTCA	382	2992-3011	3'-UTR
10	104788	TACCCCCGGTCTCCAAATAA	383	3101-3120	3'-UTR
	104789	AACATAAATAGAGGGAGCTG	384	3024-3043	3'-UTR
	104790	TTGGGTCCCCCAGGGATACCC	385	3116-3135	3'-UTR
	104791	ATAATCACAAAGTGCAAACAT	386	3039-3058	3'-UTR
	104792	AAGGCAGCTCCTACATTGGG	387	3131-3150	3'-UTR
15	104793	CGGTCTCCAAATAAATACA	388	3096-3115	3'-UTR
	104794	AAACATGTCTGAGCCAAGGC	389	3146-3165	3'-UTR
	104795	TCCCCCAGGATACCCCGGTC	390	3111-3130	3'-UTR
	104796	AGCTCCTACATTGGGTCCCC	391	3126-3145	3'-UTR
	104797	CTCCGTTTCACGGAAAACA	37	3161-3180	3'-UTR
20	104798	TGTCTGAGCCAAGGCAGCTC	392	3141-3160	3'-UTR
	104799	CAGCCTATTGTTCAGCTCCG	393	3176-3195	3'-UTR
	104800	AGAAGGCACAGAGGCCAGGG	394	3209-3228	3'-UTR
	104801	TTTCACGGAAAACATGTCT	395	3156-3175	3'-UTR
	104802	TATTGTTCAGCTCCGTTTC	396	3171-3190	3'-UTR
25	104803	AAAAACATAATCAAAAGAAG	397	3224-3243	3'-UTR
	104804	CAGATAAATATTTAAAAAA	398	3239-3258	3'-UTR
	104805	TACATGGAACAGCCTATTG	399	3186-3205	3'-UTR
	104806	TTTAGACAACCTTAATCAGAT	400	3254-3273	3'-UTR
	104807	CATAATCAAAAGAAGGCACA	401	3219-3238	3'-UTR

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	104808	ACCAAATCAGCATTGTTAG	402	3269-3288	3'-UTR
	104809	AAATATTTAAAAAACATAA	403	3234-3253	3'-UTR
	104810	GAGTGACAGTTGGTCACCAA	404	3284-3303	3'-UTR
	104811	ACAACCTTAATCAGATAAATA	405	3249-3268	3'-UTR
5	104812	CAGAGGCTCAGCAATGAGTG	406	3299-3318	3'-UTR
	104813	ATCAGCATTGTTAGACAAAC	407	3264-3283	3'-UTR
	104814	AGGGCGATTACAGACACAAAC	408	3331-3350	3'~UTR
	104815	ACAGTTGGTCACCAAATCAG	409	3279-3298	3'-UTR
	104816	TCGCCACTGAATAGTAGGGC	410	3346-3365	3'-UTR
10	104817	GCTCAGCAATGAGTGACAGT	411	3294-3313	3'-UTR
	104818	AGCAAACTTATTCTCGCC	412	3361-3380	3'-UTR
	104819	GATTACAGACACAACCCCC	413	3326-3345	3'-UTR
	104820	ACTGAATAGTAGGGCGATT	414	3341-3360	3'-UTR
	104821	ACTTTATTCTCGCCACTGA	415	3356-3375	3'-UTR
15	104822	GCTGTCTTGCTGAGGGAGC	416	0626-0645	5'-UTR
	104823	CTTAGCTGGTCCTCTGCTGT	417	0641-0660	5'-UTR
	104824	GTTGCTCTCTCCCTCTTAG	418	0656-0675	5'-UTR
	104825	TGGCGTCTGAGGGTTGTTT	419	0691-0710	5'-UTR
	104826	AGAGAACCTGCCTGGCAGCT	420	0723-0742	5'-UTR
20	104827	CAGTATGTGAGAGGAAGAGA	421	0738-0757	5'-UTR
	104828	GGTGAAGCCGTGGGTCAAGTA	422	0753-0772	5'-UTR
	104829	AGTGCTCATGGTGTCCCTTC	423	0785-0804	AUG
	104830	CCGGATCATGCTTCAGTGC	424	0800-0819	coding
	104831	GGCCAGCTCCACGTCCCGGA	425	0815-0834	coding
25	104832	GGCCCCCTGTCTTCTTGGG	426	0847-0866	coding
	104833	GGCTGAGGAACAAAGCACCGC	427	0879-0898	coding
	104834	TCAGGAAGGAGAACAGGGCTG	428	0894-0913	coding
	104835	TGGCGCCTGCCACGATCAGG	429	0909-0918	coding
	104836	GGCAGAAGAGCGTGGTGGCG	430	0924-0943	coding

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	104837	CTCCAAAGTGCAGCAGGCAG	431	0939-0958	coding
	104838	GCTGATTAGAGAGAGGTCCC	432	1596-1615	coding
	104839	TGCCTGGGCCAGAGGGCTGA	433	1611-1630	coding
	104840	GCTGCCCTCAGCTTGAGGG	434	2175-2194	coding
5	104841	GGTCAGCCACTGGAGCTGC	435	2190-2209	coding
	104842	GGGCATTGGCCCGGCGGTTC	436	2205-2224	coding
	104843	CGCCATTGCCAGGAGGGCA	437	2220-2239	coding
	104844	TATCTCTCAGCTCACGCCA	438	2235-2254	coding
	104845	GCACCACCAAGCTGGTTATCT	439	2250-2269	coding
10	104846	ACAGGCCCTCTGATGGCACC	440	2265-2284	coding
	104847	GGGAGTAGATGAGGTACAGG	441	2280-2299	coding
	104848	CCTTGAAGAGGACCTGGGAG	442	2295-2314	coding
	104849	GAGGAGCACATGGGTGGAGG	443	2327-2346	coding
	104850	GCTGATGGTGTGGGTGAGGA	444	2342-2361	coding
15	104851	GGAGACGGCGATGCGGCTGA	445	2357-2376	coding
	104852	GACCTTGGTCTGGTAGGAGA	446	2372-2391	coding
	104853	GGCAGAGAGGAGGTTGACCT	447	2387-2406	coding
	104854	GCTTGGCCTCAGCCCCCTCT	23	2436-2455	coding
	104855	TGGGCTCATACCAGGGCTTG	448	2451-2470	coding
20	104856	CCCCTCCCAGATAGATGGGC	449	2466-2485	coding
	104857	TCTCCAGCTGGAAGACCCCT	92	2481-2500	coding
	104858	TGAGTCGGTCACCCCTCTCC	450	2496-2515	coding
	104859	GATTGATCTCAGCGCTGAGT	451	2511-2530	coding
	104860	CGAGATAGTCGGGCCGATTG	452	2526-2545	coding
25	104861	CAGACTCGGCAAAGTCGAGA	89	2541-2560	coding
	104862	CAAAGTAGACCTGCCAGAC	453	2556-2575	coding
	104863	ACAGGGCAATGATCCCAGAAG	454	2571-2590	stop
	104864	ATGTTCGTCCTCCTCACAGG	455	2586-2605	stop
	104865	TTTGGGAAGGTTGGATGTT	456	2601-2620	3' -UTR

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	104866	AAGAGGTTGAGGGTGTCTGA	457	2657-2676	3' -UTR
	104867	CTCTTTTGAGCCAGAAGAG	458	2672-2691	3' -UTR
	104868	CCTAAGCCCCAATTCTCTT	459	2687-2706	3' -UTR
	104869	AGCTTGGGTTCCGACCCTAA	460	2702-2721	3' -UTR
5	104870	TTGCTTAAAGTTCTAACAGCTT	461	2717-2736	3' -UTR
	104871	GAAGTGGTGGTCTTGTTGCT	462	2732-2751	3' -UTR
	104872	TGAATCCCAGGTTTCGAAGT	463	2747-2766	3' -UTR
	104873	CAGGCCACACACATTCTGAAT	464	2762-2781	3' -UTR
	104874	CAGCACTCACTGTGCAGGC	465	2777-2796	3' -UTR
10	104875	ATTCTTAGTGGTTGCCAGCA	466	2792-2811	3' -UTR
	104876	GAGGCCCCAGTTGAATTCT	467	2807-2826	3' -UTR
	104877	CCCCAGTGAGTTCTGGAGGC	468	2822-2841	3' -UTR
	104878	GATCAAAGCTGTAGGCCCA	469	2837-2856	3' -UTR
	104879	ATTCCAGATGTCAGGGATCA	470	2852-2871	3' -UTR
15	104880	CTCCCTGGTCTCCAGATTCC	471	2867-2886	3' -UTR
	104881	GGCCAGAACCAAAGGCTCCC	472	2882-2901	3' -UTR
	104882	GTCCTGCAGCATTCTGGCCA	473	2897-2916	3' -UTR
	104883	GTGAGGTCTTCTCAAGTCCT	474	2912-2931	3' -UTR
	104884	TGTGTCAATTCTAGGTGAG	475	2927-2946	3' -UTR
20	104885	GGCCTAAGGTCCACTTGTGT	476	2942-2961	3' -UTR
	104886	ATCTGGAGAGAGGAAGGCCT	477	2957-2976	3' -UTR
	104887	AGGAAGTCTGGAAACATCTG	478	2972-2991	3' -UTR
	104888	GGGCTCCGTGTCTCAAGGAA	479	2987-3006	3' -UTR
	104889	AAATAGAGGGAGCTGGCTCC	480	3019-3038	3' -UTR
25	104890	CACAAGTGCACACATAAATA	481	3034-3053	3' -UTR
	104891	TCCCAAATAAATACATTCAT	482	3091-3110	3' -UTR
	104892	CAGGATACCCCGGTCTCCCA	483	3106-3125	3' -UTR
	104893	CTACATTGGGTCCCCCAGGA	484	3121-3140	3' -UTR
	104894	GAGCCAAGGCAGCTCCTACA	485	3136-3155	3' -UTR

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104895	ACGGAAAACATGTCTGAGCC	486	3151-3170	3'-UTR
104896	TTCAGCTCCGTTTCACGGA	487	3166-3185	3'-UTR
104897	GGGAACAGCCTATTGTTCA	488	3181-3200	3'-UTR
104898	TCAAAAGAAGGCACAGAGGC	489	3214-3233	3'-UTR
5	TTTAAAAAACATAATCAA	490	3229-3248	3'-UTR
104900	TTAACAGATAAAATATTTA	491	3244-3263	3'-UTR
104901	CATTGTTAGACAACTTAAT	492	3259-3278	3'-UTR
104902	TGGTCACCAAATCAGCATTG	493	3274-3293	3'-UTR
10	GCAATGAGTGACAGTTGGTC	494	3289-3308	3'-UTR
104904	GGGAGCAGAGGCTCAGCAAT	495	3304-3323	3'-UTR
104905	ATAGTAGGGCGATTACAGAC	496	3336-3355	3'-UTR
104906	ATTTCTCGCCACTGAATAGT	497	3351-3370	3'-UTR

¹ Emboldened residues are 2'-O-methoxyethyl residues (others are 2'-deoxy-). All 2'-O-methoxyethyl cytosines and 2'-deoxy cytosines residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

² Co-ordinates from Genbank Accession No. X02910, locus name "HSTNFA", SEQ ID NO. 1.

³ This target region is an exon-intron junction and is represented in the form, for example, I1/E2, where I, followed by a number, refers to the intron number and E, followed by a number, refers to the exon number.

TABLE 32

Inhibition of Human TNF- α mRNA Expression by Chimeric
25 (deoxy gapped) Phosphorothioate Oligodeoxynucleotides

ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
basal	---	---	0.0%	---
induced	---	---	100.0%	0.0%

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	28089	69	intron 1	42.3%	57.7%
	104649	251	5'-UTR	165.6%	---
	104650	252	5'-UTR	75.8%	24.2%
	104651	253	5'-UTR	58.2%	41.8%
5	104652	254	5'-UTR	114.5%	---
	104653	255	5'-UTR	84.9%	15.1%
	104654	256	5'-UTR	80.8%	19.2%
	104655	257	5'-UTR	94.3%	5.7%
	104656	258	5'-UTR	78.4%	21.6%
	104657	259	5'-UTR	87.4%	12.6%
10	104658	260	5'-UTR	213.4%	---
	104659	261	5'-UTR	96.3%	3.7%
	104660	262	5'-UTR	153.1%	---
	104661	263	5'-UTR	90.0%	10.0%
	104662	264	5'-UTR	33.3%	66.7%
15	104663	265	5'-UTR	144.2%	---
	104664	266	AUG	76.3%	23.7%
	104665	267	AUG	185.3%	---
	104666	268	AUG	67.4%	32.6%
20	104667	269	coding	94.3%	5.7%
	104668	270	coding	63.1%	36.9%
	104669	271	coding	50.8%	49.2%
	104670	272	coding	43.7%	56.3%
	104671	273	coding	52.2%	47.8%
25	104672	274	coding	51.8%	48.2%
	104673	275	coding	102.3%	---
	104674	276	coding	135.4%	---
	104675	277	coding	83.1%	16.9%
	104676	278	coding	87.5%	12.5%
30	104677	279	coding	53.6%	46.4%

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	104678	280	coding	75.2%	24.8%
	104679	281	coding	114.0%	---
	104680	282	coding	142.5%	---
	104681	283	coding	58.5%	41.5%
5	104682	284	coding	101.9%	---
	104683	285	coding	77.1%	22.9%
	104684	286	coding	61.0%	39.0%
	104685	287	coding	65.9%	34.1%
	104686	288	E2/I2	59.2%	40.8%
10	104687	289	coding	77.0%	23.0%
	104688	290	coding	40.1%	59.9%
	104689	291	coding	78.6%	21.4%
	104690	292	coding	90.9%	9.1%
	104691	293	coding	107.6%	---
15	104692	294	coding	63.4%	36.6%
	104693	295	coding	74.1%	25.9%
	104694	296	coding	108.3%	---
	104695	297	coding	48.2%	51.8%
	104696	298	coding	120.3%	---
20	104697	299	coding	45.0%	55.0%
	104698	300	coding	77.1%	22.9%
	104699	301	coding	143.7%	---
	104700	302	coding	96.1%	3.9%
	104701	303	coding	106.8%	---
25	104702	304	coding	157.4%	---
	104703	305	coding	84.3%	15.7%
	104704	306	coding	182.8%	---
	104705	307	coding	125.1%	---
	104706	308	coding	81.8%	18.2%
30	104707	309	coding	104.8%	---

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	104708	310	coding	163.0%	---
	104709	311	coding	95.0%	5.0%
	104710	312	coding	182.1%	---
	104711	313	coding	82.1%	17.9%
5	104712	314	coding	118.1%	---
	104713	315	coding	31.1%	68.9%
	104714	316	coding	90.5%	9.5%
	104715	317	coding	96.7%	3.3%
	104716	318	coding	180.7%	---
	104717	93	coding	71.6%	28.4%
10	104718	94	coding	187.0%	---
	104719	319	coding	88.8%	11.2%
	104720	320	coding	166.5%	---
	104721	321	coding	65.0%	35.0%
	104722	322	coding	59.6%	40.4%
	104723	26	coding	90.1%	9.9%
15	104724	323	coding	88.7%	11.3%
	104725	90	coding	94.7%	5.3%
	104726	91	coding	84.1%	15.9%
	104727	324	coding	125.3%	---
	104728	325	coding	221.7%	---
	104729	326	coding	102.4%	---
20	104730	327	coding	151.6%	---
	104731	328	coding	102.2%	---
	104732	329	coding	53.2%	46.8%
	104733	330	stop	57.0%	43.0%
	104734	88	coding	119.2%	---
	104735	331	3'-UTR	71.2%	28.8%
25	104736	332	stop	79.0%	21.0%
	104737	333	3'-UTR	87.4%	12.6%

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	104738	334	stop	36.8%	63.2%
	104739	335	3' -UTR	106.0%	---
	104740	336	3' -UTR	130.9%	---
	104741	337	3' -UTR	79.2%	20.8%
5	104742	338	3' -UTR	159.0%	---
	104743	339	3' -UTR	96.1%	3.9%
	104744	340	3' -UTR	129.9%	---
	104745	341	3' -UTR	80.2%	19.8%
	104746	342	3' -UTR	168.8%	---
	104747	343	3' -UTR	89.2%	10.8%
10	104748	344	3' -UTR	103.4%	---
	104749	345	3' -UTR	89.0%	11.0%
	104750	346	3' -UTR	160.0%	---
	104751	347	3' -UTR	60.1%	39.9%
	104752	348	3' -UTR	72.4%	27.6%
	104753	349	3' -UTR	70.0%	30.0%
15	104754	350	3' -UTR	115.6%	---
	104755	351	3' -UTR	71.7%	28.3%
	104756	352	3' -UTR	91.5%	8.5%
	104757	353	3' -UTR	85.6%	14.4%
	104758	354	3' -UTR	97.6%	2.4%
	104759	355	3' -UTR	68.6%	31.4%
20	104760	356	3' -UTR	182.4%	---
	104761	357	3' -UTR	110.9%	---
	104762	358	3' -UTR	161.4%	---
	104763	359	3' -UTR	102.0%	---
	104764	360	3' -UTR	113.5%	---
	104765	361	3' -UTR	154.8%	---
25	104766	362	3' -UTR	126.4%	---
	104767	363	3' -UTR	116.1%	---

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	104768	364	3'-UTR	177.7%	---
	104769	365	3'-UTR	89.8%	10.2%
	104770	366	3'-UTR	94.3%	5.7%
	104771	367	3'-UTR	191.2%	---
5	104772	368	3'-UTR	80.3%	19.7%
	104773	369	3'-UTR	133.9%	---
	104774	34	3'-UTR	94.8%	5.2%
	104775	370	3'-UTR	80.6%	19.4%
	104776	371	3'-UTR	90.1%	9.9%
	104777	372	3'-UTR	84.7%	15.3%
10	104778	373	3'-UTR	121.3%	---
	104779	374	3'-UTR	97.8%	2.2%
	104780	375	3'-UTR	67.6%	32.4%
	104781	376	3'-UTR	141.5%	---
	104782	377	3'-UTR	96.5%	3.5%
15	104783	378	3'-UTR	153.2%	---
	104784	379	3'-UTR	85.4%	14.6%
	104785	380	3'-UTR	163.9%	---
	104786	381	3'-UTR	82.9%	17.1%
	104787	382	3'-UTR	89.7%	10.3%
20	104788	383	3'-UTR	103.9%	---
	104789	384	3'-UTR	75.8%	24.2%
	104790	385	3'-UTR	106.3%	---
	104791	386	3'-UTR	165.3%	---
	104792	387	3'-UTR	71.8%	28.2%
25	104793	388	3'-UTR	101.9%	---
	104794	389	3'-UTR	70.7%	29.3%
	104795	390	3'-UTR	68.8%	31.2%
	104796	391	3'-UTR	93.4%	6.6%
	104797	37	3'-UTR	131.7%	---

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	104798	392	3'-UTR	89.4%	10.6%
	104799	393	3'-UTR	89.6%	10.4%
	104800	394	3'-UTR	89.0%	11.0%
	104801	395	3'-UTR	196.8%	---
5	104802	396	3'-UTR	189.3%	---
	104803	397	3'-UTR	119.7%	---
	104804	398	3'-UTR	102.4%	---
	104805	399	3'-UTR	90.6%	9.4%
	104806	400	3'-UTR	89.1%	10.9%
10	104807	401	3'-UTR	152.6%	---
	104808	402	3'-UTR	96.8%	3.2%
	104809	403	3'-UTR	178.8%	---
	104810	404	3'-UTR	94.9%	5.1%
	104811	405	3'-UTR	234.4%	---
15	104812	406	3'-UTR	114.3%	---
	104813	407	3'-UTR	153.7%	---
	104814	408	3'-UTR	86.3%	13.7%
	104815	409	3'-UTR	153.9%	---
	104816	410	3'-UTR	79.9%	20.1%
20	104817	411	3'-UTR	196.5%	---
	104818	412	3'-UTR	94.3%	5.7%
	104819	413	3'-UTR	143.3%	---
	104820	414	3'-UTR	123.8%	---
	104821	415	3'-UTR	129.2%	---
25	104822	416	5'-UTR	76.6%	23.4%
	104823	417	5'-UTR	63.9%	36.1%
	104824	418	5'-UTR	22.0%	78.0%
	104825	419	5'-UTR	109.4%	---
	104826	420	5'-UTR	45.2%	54.8%
30	104827	421	5'-UTR	68.9%	31.1%

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	104828	422	5' -UTR	70.9%	29.1%
	104829	423	AUG	46.6%	53.4%
	104830	424	coding	55.0%	45.0%
	104831	425	coding	49.5%	50.5%
5	104832	426	coding	106.0%	---
	104833	427	coding	23.7%	76.3%
	104834	428	coding	91.8%	8.2%
	104835	429	coding	72.3%	27.7%
	104836	430	coding	63.4%	36.6%
10	104837	431	coding	31.0%	69.0%
	104838	432	coding	18.0%	82.0%
	104839	433	coding	67.9%	32.1%
	104840	434	coding	93.8%	6.2%
	104841	435	coding	43.0%	57.0%
15	104842	436	coding	73.2%	26.8%
	104843	437	coding	48.1%	51.9%
	104844	438	coding	39.2%	60.8%
	104845	439	coding	37.6%	62.4%
	104846	440	coding	81.7%	18.3%
20	104847	441	coding	50.8%	49.2%
	104848	442	coding	56.7%	43.3%
	104849	443	coding	51.8%	48.2%
	104850	444	coding	91.8%	8.2%
	104851	445	coding	93.9%	6.1%
25	104852	446	coding	100.9%	---
	104853	447	coding	67.7%	32.3%
	104854	23	coding	11.0%	89.0%
	104855	448	coding	62.5%	37.5%
	104856	449	coding	67.8%	32.2%
30	104857	92	coding	28.1%	71.9%

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	104858	450	coding	76.2%	23.8%
	104859	451	coding	52.3%	47.7%
	104860	452	coding	93.6%	6.4%
	104861	89	coding	79.3%	20.7%
5	104862	453	coding	63.1%	36.9%
	104863	454	stop	64.5%	35.5%
	104864	455	stop	43.2%	56.8%
	104865	456	3'-UTR	83.1%	16.9%
	104866	457	3'-UTR	49.4%	50.6%
	104867	458	3'-UTR	49.5%	50.5%
	104868	459	3'-UTR	89.6%	10.4%
10	104869	460	3'-UTR	21.4%	78.6%
	104870	461	3'-UTR	118.0%	---
	104871	462	3'-UTR	55.8%	44.2%
	104872	463	3'-UTR	49.0%	51.0%
	104873	464	3'-UTR	92.6%	7.4%
	104874	465	3'-UTR	33.4%	66.6%
	104875	466	3'-UTR	36.2%	63.8%
15	104876	467	3'-UTR	73.4%	26.6%
	104877	468	3'-UTR	40.9%	59.1%
	104878	469	3'-UTR	78.7%	21.3%
	104879	470	3'-UTR	75.4%	24.6%
	104880	471	3'-UTR	50.2%	49.8%
	104881	472	3'-UTR	47.0%	53.0%
	104882	473	3'-UTR	82.7%	17.3%
20	104883	474	3'-UTR	46.4%	53.6%
	104884	475	3'-UTR	46.1%	53.9%
	104885	476	3'-UTR	156.9%	---
	104886	477	3'-UTR	102.4%	---
	104887	478	3'-UTR	59.1%	40.9%

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	104888	479	3'-UTR	64.7%	35.3%
	104889	480	3'-UTR	83.7%	16.3%
	104890	481	3'-UTR	52.9%	47.1%
	104891	482	3'-UTR	87.9%	12.1%
5	104892	483	3'-UTR	39.8%	60.2%
	104893	484	3'-UTR	71.1%	28.9%
	104894	485	3'-UTR	34.0%	66.0%
	104895	486	3'-UTR	129.8%	---
	104896	487	3'-UTR	57.6%	42.4%
10	104897	488	3'-UTR	49.6%	50.4%
	104898	489	3'-UTR	71.7%	28.3%
	104899	490	3'-UTR	101.5%	---
	104900	491	3'-UTR	142.1%	---
	104901	492	3'-UTR	55.9%	44.1%
15	104902	493	3'-UTR	85.3%	14.7%
	104903	494	3'-UTR	46.0%	54.0%
	104904	495	3'-UTR	59.9%	40.1%
	104905	496	3'-UTR	47.2%	52.8%
	104906	497	3'-UTR	56.3%	43.7%

20 Oligonucleotides 104662 (SEQ ID NO: 264), 104669 (SEQ
 ID NO: 271), 104670 (SEQ ID NO: 272), 104688 (SEQ ID NO:
 290), 104695 (SEQ ID NO: 297), 104697 (SEQ ID NO: 299),
 104713 (SEQ ID NO: 315), 104738 (SEQ ID NO: 334), 104824
 (SEQ ID NO: 418), 104826 (SEQ ID NO: 420), 104829 (SEQ ID
 25 NO: 423), 104831 (SEQ ID NO: 425), 104833 (SEQ ID NO: 427),
 104837 (SEQ ID NO: 431), 104838 (SEQ ID NO: 432), 104841
 (SEQ ID NO: 435), 104843 (SEQ ID NO: 437), 104844 (SEQ ID
 NO: 438), 104845 (SEQ ID NO: 439), 104847 (SEQ ID NO: 441),
 104854 (SEQ ID NO: 23), 104857 (SEQ ID NO: 92), 104864 (SEQ
 30 ID NO: 455), 104866 (SEQ ID NO: 457), 104867 (SEQ ID NO:
 458), 104869 (SEQ ID NO: 460), 104872 (SEQ ID NO: 463),

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104874 (SEQ ID NO: 465), 104875 (SEQ ID NO: 466), 104877
(SEQ ID NO: 468), 104880 (SEQ ID NO: 471), 104881 (SEQ ID
NO: 472), 104883 (SEQ ID NO: 474), 104884 (SEQ ID NO: 475),
104892 (SEQ ID NO: 483), 104894 (SEQ ID NO: 485), 104897
5 (SEQ ID NO: 488), 104903 (SEQ ID NO: 494) and 104905 (SEQ
ID NO: 496) gave approximately 50% or greater reduction in
TNF- α mRNA expression in this assay. Oligonucleotides
10 104713 (SEQ ID NO: 315), 104824 (SEQ ID NO: 418), 104833
(SEQ ID NO: 427), 104837 (SEQ ID NO: 431), 104838 (SEQ ID
NO: 432), 104854 (SEQ ID NO: 23), 104857 (SEQ ID NO: 92),
and 104869 (SEQ ID NO: 460) gave approximately 70% or
greater reduction in TNF- α mRNA expression in this assay.

**EXAMPLE 23: Dose response of chimeric (deoxy gapped)
antisense phosphorothioate oligodeoxynucleotide effects on**

15 **TNF- α mRNA and protein levels**

Several oligonucleotides from the initial screen were chosen for dose response assays. NeoHk cells were grown, treated and processed as described in Example 3.

20 LIPOFECTIN® was added at a ratio of 3 μ g/ml per 100 nM of oligonucleotide. The control included LIPOFECTIN® at a concentration of 9 μ g/ml.

The human promonocytic leukaemia cell line, THP-1 (American Type Culture Collection, Manassas, VA) was maintained in RPMI 1640 growth media supplemented with 10% fetal calf serum (FCS; Life Technologies, Rockville, MD). A total of 8×10^5 cells were employed for each treatment by combining 50 μ l of cell suspension in OPTIMEM™, 1% FBS with oligonucleotide at the indicated concentrations to reach a final volume of 100 μ l with OPTIMEM™, 1% FBS. Cells were 30 then transferred to a 1 mm electroporation cuvette and electroporated using an Electrocell Manipulator 600 instrument (Biotechnologies and Experimental Research, Inc.) employing 90 V, 1000 μ F, at 13 Ω . Electroporated

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cells were then transferred to 24 well plates. 400 µl of RPMI 1640, 10% FCS was added to the cells and the cells were allowed to recover for 6 hrs. Cells were then induced with LPS at a final concentration of 100 ng/ml for 2 hours.

5 RNA was isolated and processed as described in Example 3.

Results with NeoHK cells are shown in Table 33 for mRNA, and Table 34 for protein. Results with THP-1 cells are shown in Table 35.

Most of the oligonucleotides tested showed dose 10 response effects with a maximum inhibition of mRNA greater than 70% and a maximum inhibition of protein greater than 85%.

TABLE 33

Dose Response of NeoHK Cells to TNF- α

15 Chimeric (deoxy gapped) Antisense Oligonucleotides

ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% mRNA Expression	% mRNA Inhibition
induced	---	---	---	100%	---
16798	128	coding	30 nM	87%	13%
"	"	"	100 nM	129%	---
20	"	"	300 nM	156%	---
21823	69	intron 1	30 nM	82%	18%
"	"	"	100 nM	90%	10%
"	"	"	300 nM	59%	41%
28088	68	intron 1	30 nM	68%	32%
25	"	"	100 nM	43%	57%
"	"	"	300 nM	42%	58%
28089	69	intron 1	30 nM	59%	41%
"	"	"	100 nM	44%	56%
"	"	"	300 nM	38%	62%
30	104697	299	coding	30 nM	60%
"	"	"	100 nM	45%	55%

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	"	"	"	300 nM	27%	73%
104777	372	3'-UTR		30 nM	66%	34%
	"	"	"	100 nM	55%	45%
	"	"	"	300 nM	43%	57%

5

TABLE 34**Dose Response of NeoHK Cells to TNF- α** **Chimeric (deoxy gapped) Antisense Oligonucleotides**

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% Protein Expression	% Protein Inhibition
	induced	---	---	---	100.0%	---
10	16798	128	coding	30 nM	115.0%	---
	"	"	"	100 nM	136.0%	---
	"	"	"	300 nM	183.0%	---
15	28089	69	intron 1	30 nM	87.3%	12.7%
	"	"	"	100 nM	47.4%	52.6%
	"	"	"	300 nM	22.8%	77.2%
20	104681	283	coding	30 nM	91.3%	8.7%
	"	"	"	100 nM	62.0%	38.0%
	"	"	"	300 nM	28.5%	71.5%
25	104697	299	coding	30 nM	87.1%	12.9%
	"	"	"	100 nM	59.6%	40.4%
	"	"	"	300 nM	29.1%	70.9%
30	104838	432	coding	30 nM	91.9%	8.1%
	"	"	"	100 nM	56.9%	43.1%
	"	"	"	300 nM	14.8%	85.2%
35	104854	23	coding	30 nM	64.4%	35.6%
	"	"	"	100 nM	42.3%	57.7%
	"	"	"	300 nM	96.1%	3.9%
40	104869	460	3'-UTR	30 nM	88.9%	11.1%
	"	"	"	100 nM	56.8%	43.2%

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"	"	"	300 nM	42.3%	57.7%
---	---	---	--------	-------	-------

TABLE 35

Dose Response of LPS-Induced THP-1 Cells to Chimeric (deoxy
gapped) TNF- α Antisense Phosphorothioate
5 Oligodeoxynucleotides (ASOs)

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% mRNA Expression	% mRNA Inhibition
	induced	---	---	---	100%	---
	16798	128	coding	1 μ M	102%	--
	"	"	"	3 μ M	87%	13%
10	"	"	"	10 μ M	113%	---
	"	"	"	30 μ M	134%	---
	28089	69	intron 1	1 μ M	39%	61%
	"	"	"	3 μ M	79%	21%
	"	"	"	10 μ M	91%	9%
15	"	"	"	30 μ M	63%	37%
	104697	299	coding	1 μ M	99%	1%
	"	"	"	3 μ M	96%	4%
	"	"	"	10 μ M	92%	8%
	"	"	"	30 μ M	52%	48%
20	104838	432	coding	1 μ M	31%	69%
	"	"	"	3 μ M	20%	80%
	"	"	"	10 μ M	15%	85%
	"	"	"	30 μ M	7%	93%
	104854	23	coding	1 μ M	110%	---
25	"	"	"	3 μ M	90%	10%
	"	"	"	10 μ M	95%	5%
	"	"	"	30 μ M	61%	39%

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EXAMPLE 24: Further Optimization of Human TNF- α Antisense Oligonucleotide Chemistry

Additional analogs of TNF- α oligonucleotides were designed and synthesized to find an optimum gap size. The 5 sequences and chemistries are shown in Table 36.

Dose response experiments are performed as described in Example 3.

TABLE 36
Nucleotide Sequences of TNF- α Chimeric Backbone (deoxy
10 **gapped) Oligonucleotides**

	ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET NUCLEOTIDE CO- ORDINATES ²	GENE TARGET REGION
	110554	GCTGATTAGAGAGAGGTCCC	432	104838	analog
	110555	GCTGATTAGAGAGAGGTCCC	"	"	
15	110556	GCTGATTAGAGAGAGGTCCC	"	"	
	110557	GCTGATTAGAGAGAGGTCCC	"	"	
	110583	GCTGATTAGAGAGAGGTCCC	"	"	
	110558	CTGATTAGAGAGAGGTCCC	498	1596-1614	coding
	110559	CTGATTAGAGAGAGGTCCC	"	"	"
20	110560	CTGATTAGAGAGAGGTCCC	"	"	"
	110561	CTGATTAGAGAGAGGTCCC	"	"	"
	110562	CTGATTAGAGAGAGGTCCC	"	"	"
	110563	CTGATTAGAGAGAGGTCCC	"	"	"
	110564	CTGATTAGAGAGAGGTCCC	"	"	"
25	110565	CTGATTAGAGAGAGGTCCC	"	"	"
	110566	CTGATTAGAGAGAGGTCCC	"	"	"
	110567	CTGATTAGAGAGAGGTCCC	"	"	"
	110584	CTGATTAGAGAGAGGTCCC	"	"	"
	108371	CTGATTAGAGAGAGGTCC	499	1597-1614	coding

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	110568	CTGATTAGAGAGAGGTCC	"	"	"
	110569	CTGATTAGAGAGAGGTCC	"	"	"
	110570	CTGATTAGAGAGAGGTCC	"	"	"
	110585	CTGATTAGAGAGAGGTCC	"	"	"
5	110571	CTGGTTATCTCTCAGCTCCA	299	104697	analog
	110572	CTGGTTATCTCTCAGCTCCA	"	"	
	110573	CTGGTTATCTCTCAGCTCCA	"	"	
	110586	CTGGTTATCTCTCAGCTCCA	"	"	
	110574	GATCACTCCAAAGTGCAGCA	283	104681	analog
10	110575	GATCACTCCAAAGTGCAGCA	"	"	
	110576	GATCACTCCAAAGTGCAGCA	"	"	
	110587	GATCACTCCAAAGTGCAGCA	"	"	
	110577	AGCTTGGGTTCCGACCTAA	460	104689	analog
	110578	AGCTTGGGTTCCGACCTAA	"	"	
15	110579	AGCTTGGGTTCCGACCTAA	"	"	
	110588	AGCTTGGGTTCCGACCTAA	"	"	
	110580	AGGTTGACCTTGGTCTGGTA	315	104713	analog
	110581	AGGTTGACCTTGGTCTGGTA	"	"	
	110582	AGGTTGACCTTGGTCTGGTA	"	"	
20	110589	AGGTTGACCTTGGTCTGGTA	"	"	
	110637	GTGTGCCAGACACCCCTATCT	69	21823	analog
	110651	GTGTGCCAGACACCCCTATCT	"	"	
	110665	GTGTGCCAGACACCCCTATCT	"	"	
	110679	GTGTGCCAGACACCCCTATCT	"	"	
25	110693	GTGTGCCAGACACCCCTATCT	"	"	
	110707	GTGTGCCAGACACCCCTATCT	"	"	
	110590	TGAGTGTCTTCTGTGTGCCA	500	1411-1430	intron 1
	110597	TGAGTGTCTTCTGTGTGCCA	"	"	"
	110604	TGAGTGTCTTCTGTGTGCCA	"	"	"

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	110611	TGAGTGTCTTCTGTGTGCCA	"	"	"
	110618	TGAGTGTCTTCTGTGTGCCA	"	"	"
	110625	TGAGTGTCTTCTGTGTGCCA	"	"	"
	110591	GAGTGTCTTCTGTGTGCCAG	501	1410-1429	intron 1
5	110598	GAGTGTCTTCTGTGTGCCAG	"	"	"
	110605	GAGTGTCTTCTGTGTGCCAG	"	"	"
	110612	GAGTGTCTTCTGTGTGCCAG	"	"	"
	110619	GAGTGTCTTCTGTGTGCCAG	"	"	"
	110626	GAGTGTCTTCTGTGTGCCAG	"	"	"
10	110592	AGTGTCTTCTGTGTGCCAGA	144	100181	analog
	110599	AGTGTCTTCTGTGTGCCAGA	"	"	
	110606	AGTGTCTTCTGTGTGCCAGA	"	"	
	110613	AGTGTCTTCTGTGTGCCAGA	"	"	
	110620	AGTGTCTTCTGTGTGCCAGA	"	"	
15	110627	AGTGTCTTCTGTGTGCCAGA	"	"	
	110593	GTGTCTTCTGTGTGCCAGAC	145	100182	analog
	110600	GTGTCTTCTGTGTGCCAGAC	"	"	
	110607	GTGTCTTCTGTGTGCCAGAC	"	"	
	110614	GTGTCTTCTGTGTGCCAGAC	"	"	
20	110621	GTGTCTTCTGTGTGCCAGAC	"	"	
	110628	GTGTCTTCTGTGTGCCAGAC	"	"	
	110594	TGTCTTCTGTGTGCCAGACA	146	100183	analog
	110601	TGTCTTCTGTGTGCCAGACA	"	"	
	110608	TGTCTTCTGTGTGCCAGACA	"	"	
25	110615	TGTCTTCTGTGTGCCAGACA	"	"	
	110622	TGTCTTCTGTGTGCCAGACA	"	"	
	110629	TGTCTTCTGTGTGCCAGACA	"	"	
	110595	GTCTTCTGTGTGCCAGACAC	147	100184	analog
	110602	GTCTTCTGTGTGCCAGACAC	"	"	

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110609	GTCTTCTGTGTGCCAGACAC	"	"
110616	GTCTTCTGTGTGCCAGACAC	"	"
110623	GTCTTCTGTGTGCCAGACAC	"	"
110630	GTCTTCTGTGTGCCAGACAC	"	"
5	TCTTCTGTGTGCCAGACACC	148	100185 analog
	TCTTCTGTGTGCCAGACACC	"	"
10	TCTTCTGTGTGCCAGACACC	"	"
	CTTCTGTGTGCCAGACACCC	149	100186 analog
	CTTCTGTGTGCCAGACACCC	"	"
	CTTCTGTGTGCCAGACACCC	"	"
	CTTCTGTGTGCCAGACACCC	"	"
15	CTTCTGTGTGCCAGACACCC	"	"
	CTTCTGTGTGCCAGACACCC	"	"
	TTCTGTGTGCCAGACACCC	150	100187 analog
	TTCTGTGTGCCAGACACCC	"	"
	TTCTGTGTGCCAGACACCC	"	"
20	TTCTGTGTGCCAGACACCC	"	"
	TTCTGTGTGCCAGACACCC	"	"
	TTCTGTGTGCCAGACACCC	"	"
	TCTGTGTGCCAGACACCTA	151	100188 analog
	TCTGTGTGCCAGACACCTA	"	"
25	TCTGTGTGCCAGACACCTA	"	"
	CTGTGTGCCAGACACCTAT	152	100189 analog

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	110649	CTGTGTGCCAGACACCCCTAT	"	"
	110663	CTGTGTGCCAGACACCCCTAT	"	"
	110677	CTGTGTGCCAGACACCCCTAT	"	"
	110691	CTGTGTGCCAGACACCCCTAT	"	"
5	110705	CTGTGTGCCAGACACCCCTAT	"	"
	110636	TGTGTGCCAGACACCCCTATC	153	100190 analog
	110650	TGTGTGCCAGACACCCCTATC	"	"
	110664	TGTGTGCCAGACACCCCTATC	"	"
	110678	TGTGTGCCAGACACCCCTATC	"	"
10	110692	TGTGTGCCAGACACCCCTATC	"	"
	110706	TGTGTGCCAGACACCCCTATC	"	"
	110638	TGTGCCAGACACCCCTATCTT	154	100191 analog
	110652	TGTGCCAGACACCCCTATCTT	"	"
	110666	TGTGCCAGACACCCCTATCTT	"	"
15	110680	TGTGCCAGACACCCCTATCTT	"	"
	110694	TGTGCCAGACACCCCTATCTT	"	"
	110708	TGTGCCAGACACCCCTATCTT	"	"
	110639	GTGCCAGACACCCCTATCTTC	155	100192 analog
	110653	GTGCCAGACACCCCTATCTTC	"	"
20	110667	GTGCCAGACACCCCTATCTTC	"	"
	110681	GTGCCAGACACCCCTATCTTC	"	"
	110695	GTGCCAGACACCCCTATCTTC	"	"
	110709	GTGCCAGACACCCCTATCTTC	"	"
	110640	TGCCAGACACCCCTATCTTCT	156	100193 analog
25	110654	TGCCAGACACCCCTATCTTCT	"	"
	110668	TGCCAGACACCCCTATCTTCT	"	"
	110682	TGCCAGACACCCCTATCTTCT	"	"
	110696	TGCCAGACACCCCTATCTTCT	"	"
	110710	TGCCAGACACCCCTATCTTCT	"	"

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	110641	GCCAGACACCCTATCTTCTT	157	100194 analog
	110655	GCCAGACACCCTATCTTCTT	"	"
	110669	GCCAGACACCCTATCTTCTT	"	"
	110683	GCCAGACACCCTATCTTCTT	"	"
5	110697	GCCAGACACCCTATCTTCTT	"	"
	110711	GCCAGACACCCTATCTTCTT	"	"
	110642	CCAGACACCCTATCTTCTTC	158	100195 analog
	110656	CCAGACACCCTATCTTCTTC	"	"
	110670	CCAGACACCCTATCTTCTTC	"	"
10	110684	CCAGACACCCTATCTTCTTC	"	"
	110698	CCAGACACCCTATCTTCTTC	"	"
	110712	CCAGACACCCTATCTTCTTC	"	"
	110643	CAGACACCCTATCTTCTTC	159	100196 analog
	110657	CAGACACCCTATCTTCTTC	"	"
15	110671	CAGACACCCTATCTTCTTC	"	"
	110685	CAGACACCCTATCTTCTTC	"	"
	110699	CAGACACCCTATCTTCTTC	"	"
	110713	CAGACACCCTATCTTCTTC		"
	110644	AGACACCCTATCTTCTTC	160	100197 analog
20	110658	AGACACCCTATCTTCTTC	"	"
	110672	AGACACCCTATCTTCTTC	"	"
	110686	AGACACCCTATCTTCTTC	"	"
	110700	AGACACCCTATCTTCTTC	"	"
	110714	AGACACCCTATCTTCTTC	"	"
25	110645	GACACCCTATCTTCTTC	161	100198 analog
	110659	GACACCCTATCTTCTTC	"	"
	110673	GACACCCTATCTTCTTC	"	"
	110687	GACACCCTATCTTCTTC	"	"

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110701 GACACCCTATCTTCTTCTCT " "
110715 GACACCCTATCTTCTTCTCT " "

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethoxy cytidines and 2'-
5 deoxycytidines are 5-methyl-cytidines; all linkages are phosphorothioate linkages.

²Co-ordinates from Genbank Accession No. X02910, locus name "HSTNFA", SEQ ID NO. 1.

Example 25: Effect of TNF- α antisense oligonucleotides in

10 **TNF- α transgenic mouse models**

The effect of TNF- α antisense oligonucleotides is studied in transgenic mouse models of human diseases. Such experiments can be performed through contract laboratories (e.g. The Laboratory of Molecular Genetics at The Hellenic 15 Pasteur Institute, Athens, Greece) where such transgenic mouse models are available. Such models are available for testing human oligonucleotides in arthritis (Keffer, J., et al., *EMBO J.*, 1991, 10, 4025-4031) and multiple sclerosis (Akassoglou, K., et al., *J. Immunol.*, 1997, 158, 438-445) 20 models. A model for inflammatory bowel disease is available for testing mouse oligonucleotides (Kontoyiannis, D., et al., *Immunity*, 1999, 10, 387-398).

Briefly, litters of the appropriate transgenic mouse strain are collected and weighed individually. Twice 25 weekly from birth, oligonucleotide in saline is administered intraperitoneally or intravenously. Injections continue for 7 weeks. Each week the animals are scored for manifestations of the appropriate disease. After the final treatment, the mice are sacrificed and 30 histopathology is performed for indicators of disease as indicated in the references cited for each model.

What is claimed is:

1. An oligonucleotide 8 to 30 nucleotides in length comprising a nucleotide sequence complementary to an intron of a nucleic acid encoding human tumor necrosis factor- α ,
5 wherein said oligonucleotide inhibits the expression of said human tumor necrosis factor- α .

2. The oligonucleotide of claim 1 wherein said intron is intron 1 of a nucleic acid encoding human tumor
10 necrosis factor- α .

3. The oligonucleotide of claim 2 comprising SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:149 or SEQ ID NO:157.

4. The oligonucleotide of claim 1 wherein said intron is intron 2 of a nucleic acid encoding human tumor
15 necrosis factor- α .

5. The oligonucleotide of claim 1 wherein said intron is intron 3 of a nucleic acid encoding human tumor necrosis factor- α .

6. The oligonucleotide of claim 5 comprising SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82 or SEQ ID NO:84.
20

7. The oligonucleotide of claim 1 which contains at least one phosphorothioate intersugar linkage.

8. The oligonucleotide of claim 1 which has at least
25 one 2'-O-methoxyethyl modification.

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9. The oligonucleotide of claim 1 which contains at least one 5-methyl cytidine.

10. The oligonucleotide of claim 8 in which every 2'-O-methoxyethyl modified cytidine residue is a 5-methyl
5 cytidine.

11. The oligonucleotide of claim 9 in which every cytidine residue is a 5-methyl cytidine.

12. The oligonucleotide of claim 1 which contains at least one methylene(methylimino) intersugar linkage.

10 13. A composition comprising the oligonucleotide of claim 1 and a pharmaceutically acceptable carrier or diluent.

14. The composition of claim 13 wherein said pharmaceutically acceptable carrier or diluent comprises a
15 lipid or liposome.

15. A method of modulating the expression of human tumor necrosis factor- α in cells or tissue comprising contacting said cells or tissue with the oligonucleotide of claim 1.

20 16. A method of reducing an inflammatory response of human cells comprising contacting said human cells with the oligonucleotide of claim 1.

17. A method of treating a human having a disease or condition associated with tumor necrosis factor- α

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comprising administering to said animal a therapeutically or prophylactically effective amount of an oligonucleotide of claim 1.

18. The method of claim 17 wherein said administering 5 is through oral delivery.

19. The method of claim 17 wherein the disease or condition is associated with overexpression of tumor necrosis factor- α .

20. The method of claim 19 wherein said disease or 10 condition is an inflammatory or autoimmune disease or condition.

21. The method of claim 20 wherein said inflammatory or autoimmune disease or condition is diabetes, 15 inflammatory bowel disease, multiple sclerosis, pancreatitis, rheumatoid arthritis, hepatitis, atopic dermatitis or allograft rejection.

22. The method of claim 19 wherein said disease or condition is an infectious disease.

23. The method of claim 22 wherein said infectious 20 disease is hepatitis.

24. An oligonucleotide complementary to a nucleic acid molecule encoding human tumor necrosis factor- α , wherein said oligonucleotide inhibits the expression of said human tumor necrosis factor- α and comprises SEQ ID 25 NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:39, SEQ ID NO:88, SEQ ID NO:90, SEQ ID

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NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID
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NO:271, SEQ ID NO:272, SEQ ID NO:290, SEQ ID NO:297, SEQ ID
NO:299, SEQ ID NO: 315, SEQ ID NO:334, SEQ ID NO:418, SEQ
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ID NO:465, SEQ ID NO:466, SEQ ID NO:468, SEQ ID NO:471, SEQ
10 ID NO:472, SEQ ID NO:474, SEQ ID NO:475, SEQ ID NO:483, SEQ
ID NO:485, SEQ ID NO:488, SEQ ID NO:494 or SEQ ID NO:496.

25. The oligonucleotide of claim 24 which contains at least one phosphorothioate intersugar linkage.

26. The oligonucleotide of claim 24 which has at 15 least one 2'-O-methoxyethyl modification.

27. The oligonucleotide of claim 24 which contains at least one 5-methyl cytidine.

28. The oligonucleotide of claim 26 in which every 2'-O-methoxyethyl modified cytidine residue is a 5-methyl 20 cytidine.

29. The oligonucleotide of claim 27 in which every cytidine residue is a 5-methyl cytidine.

30. The oligonucleotide of claim 24 which contains at least one methylene(methylimino) intersugar linkage.

25 31. A composition comprising the oligonucleotide of claim 24 and a pharmaceutically acceptable carrier or diluent.

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32. The composition of claim 31 wherein said pharmaceutically acceptable carrier or diluent comprises a lipid or liposome.

33. A method of modulating the expression of human
5 tumor necrosis factor- α in cells or tissue comprising
contacting said cells or tissue with the oligonucleotide of
claim 24.

34. A method of reducing an inflammatory response of
10 human cells comprising contacting said human cells with the
oligonucleotide of claim 24.

35. A method of treating a human having a disease or
condition associated with tumor necrosis factor- α
comprising administering to said animal a therapeutically
15 or prophylactically effective amount of an oligonucleotide
of claim 24.

36. The method of claim 35 wherein said administering
is through oral delivery.

37. The method of claim 35 wherein the disease or
20 condition is associated with overexpression of tumor
necrosis factor- α .

38. The method of claim 37 wherein said disease or
condition is an inflammatory or autoimmune disease or
condition.

25 39. The method of claim 38 wherein said inflammatory
or autoimmune disease or condition is diabetes,
inflammatory bowel disease, multiple sclerosis,

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pancreatitis, rheumatoid arthritis, atopic dermatitis or allograft rejection.

40. The method of claim 39 wherein said disease or condition is an infectious disease.

5 41. The method of claim 40 wherein said infectious disease is hepatitis.

42. An oligonucleotide 8 to 30 nucleotides in length comprising a nucleotide sequence complementary to a nucleic acid encoding human tumor necrosis factor- α , wherein said 10 oligonucleotide inhibits the expression of said human tumor necrosis factor- α , and has at least one 2'-O-methoxyethyl modification.

43. An oligonucleotide 8 to 30 nucleotides in length comprising a nucleotide sequence complementary to a nucleic acid encoding human tumor necrosis factor- α , wherein said 15 oligonucleotide inhibits the expression of said human tumor necrosis factor- α , and contains at least one 5-methyl cytidine.

44. The oligonucleotide of claim 42 in which every 20 2'-O-methoxyethyl modified cytidine residue is a 5-methyl cytidine.

45. The oligonucleotide of claim 43 in which every cytidine residue is a 5-methyl cytidine.

46. An oligonucleotide 8 to 30 nucleotides in length 25 comprising a nucleotide sequence complementary to a nucleic acid encoding human tumor necrosis factor- α , wherein said oligonucleotide inhibits the expression of said human tumor

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necrosis factor- α , and which contains at least one methylene(methylimino) intersugar linkage.

47. An antisense oligonucleotide capable of modulating gene expression in adipose tissue.

5

48. The antisense oligonucleotide of claim 47 which is targeted to human tumor necrosis factor- α .

49. A method of modulating the expression of a selected gene product in adipose tissue comprising
10 contacting said adipose tissue with an antisense compound targeted to said selected gene.

50. The method of claim 49 wherein said selected gene product is tumor necrosis factor- α .

51. A method of modulating the function of a selected nucleic acid sequence in adipose tissue comprising
15 contacting said adipose tissue with an antisense compound targeted to said selected nucleic acid sequence.

52. The method of claim 51 wherein said selected nucleic acid sequence encodes tumor necrosis factor- α .

20 53. A method of treating a human having a disease or condition associated with expression of a selected nucleic acid sequence in adipose tissue comprising administering to said animal a therapeutically or prophylactically effective amount of an antisense compound targeted to said selected
25 nucleic acid sequence.

54. The method of claim 53 wherein said administering is through oral delivery.

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55. A method of reducing the blood glucose level in a human comprising administering to said animal a therapeutically or prophylactically effective amount of an oligonucleotide of claim 1.

5 56. A method of reducing the blood glucose level in a human comprising administering to said animal a therapeutically or prophylactically effective amount of an oligonucleotide of claim 24.

SEQUENCE LISTING

<110> Baker, Brenda
Bennett, C. Frank
Butler, Madeline M.
Shanahan, William R.
Isis Pharmaceuticals, Inc.

<120> ANTISENSE OLIGONUCLEOTIDE MODULATION OF TUMOR NECROSIS FACTOR- α (TNF- α) EXPRESSION

<130> ISPH-0409

<150> 09/313,932
<151> 1999-05-18

<150> 09/166,168
<151> 1998-10-05

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<301> Nedwin, G.E.
Naylor, S.L.
Sakaguchi, A.Y.
Smith, D.
Jarrett-Nedwin, J.
Pennica, D.
Goeddel, D.V.
Gray, P.W.
<302> Human lymphotoxin and tumor necrosis factor genes: structure, homology and chromosomal localization
<303> Nucleic Acids Res.
<304> 13
<305> 17
<306> 6361-6373
<307> 1985-09-11
<308> X02910 Genbank
<309> 1997-02-17

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 Phe Pro Arg Asp Leu Ser Leu Ile Ser Pro Leu
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His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln			
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Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu			
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Shakhov, A.N.
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/23205

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68; C07H 21/04; A61K 48/00; C12N 15/00, 15/85

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.21, 91.1, 91.4, 325, 366, 375; 536/23.1, 24.3, 24.5; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

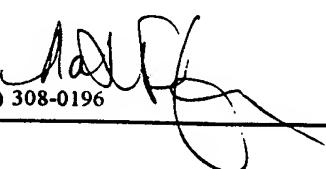
APS (US AND FOREIGN PATENTS), DIALOG (MEDLINE, BIOSIS)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/40162 A1 (EAST CAROLINA UNIVERSITY) 19 December 1996, see entire document.	1, 5-7, 12
X	WO 95/23225 A1 (RIBOZYME PHARMACEUTICALS, INC.) 31 August 1995, see entire document.	24-25, 33-38
Y	BRANCH, A. D. A good antisense molecule is hard to find. TIBS. February 1998, Vol. 23, pages 45-50, see entire document.	1-56

 Further documents are listed in the continuation of Box C. See patent family annex.

- Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
02 NOVEMBER 1999	18 NOV 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer MARY SCHMIDT Telephone No. (703) 308-0196
Facsimile No. (703) 305-3230	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23205

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 7.21, 91.1, 91.4, 325, 366, 375; 536/23.1, 24.3, 24.5; 514/44